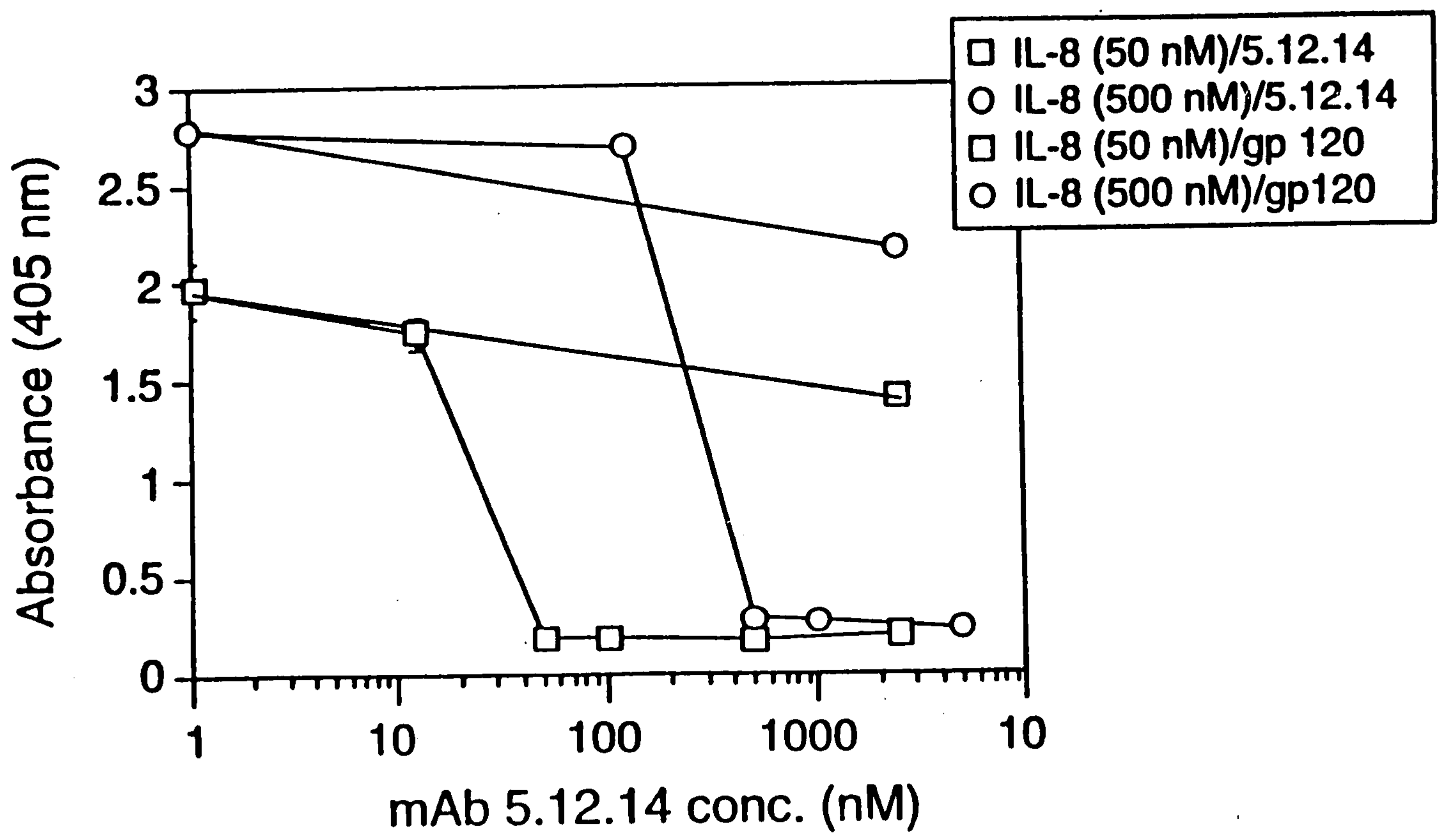
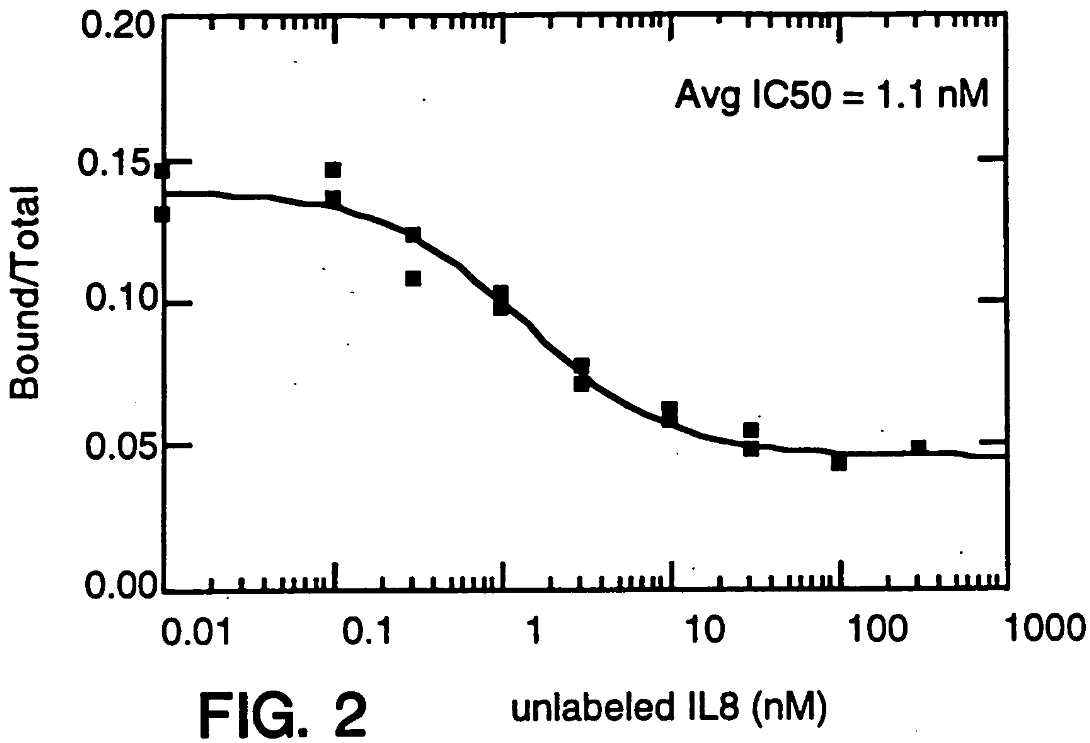
FIG. 1

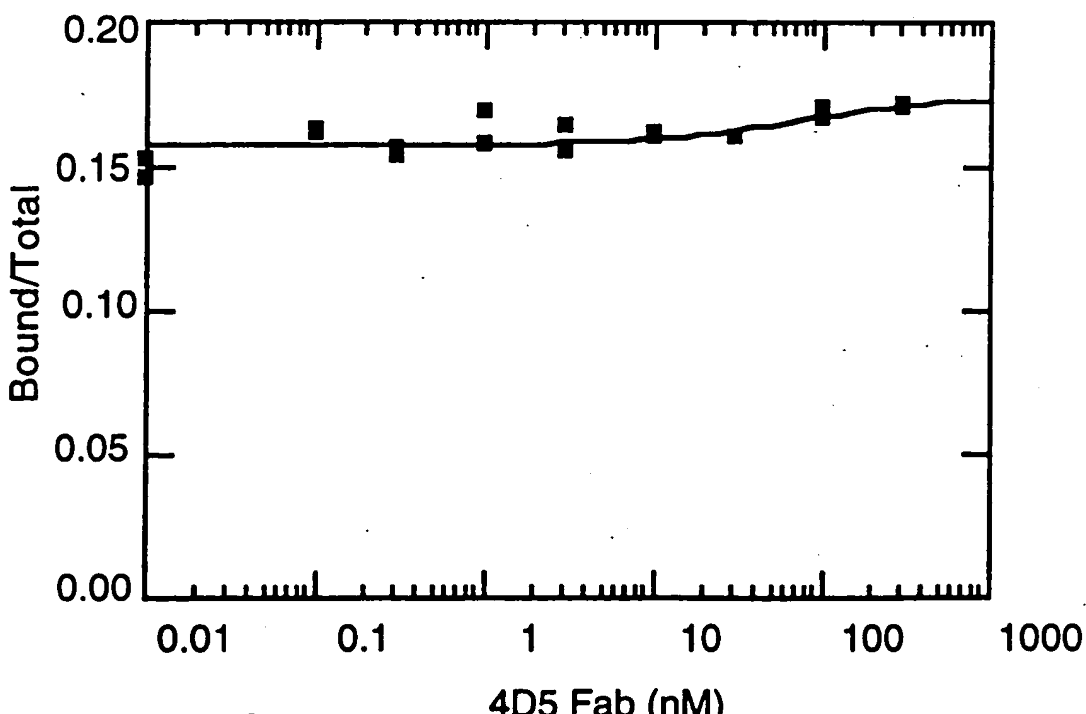


IL-8 (50 nM)/5.12.14
IL-8 (500 nM)/5.12.14
IL-8 (50 nM)/gp 120
IL-8 (500 nM)/gp120
Absorbance (405 nm)
3
2.5
2
1.5
1
0.5
0
1
10
100
1000
10
mAb 5.12.14 conc. (nM)



Avg IC50 = 1.1 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
unlabeled IL8 (nM)

FIG. 2



Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
4D5 Fab (nM)

FIG. 3

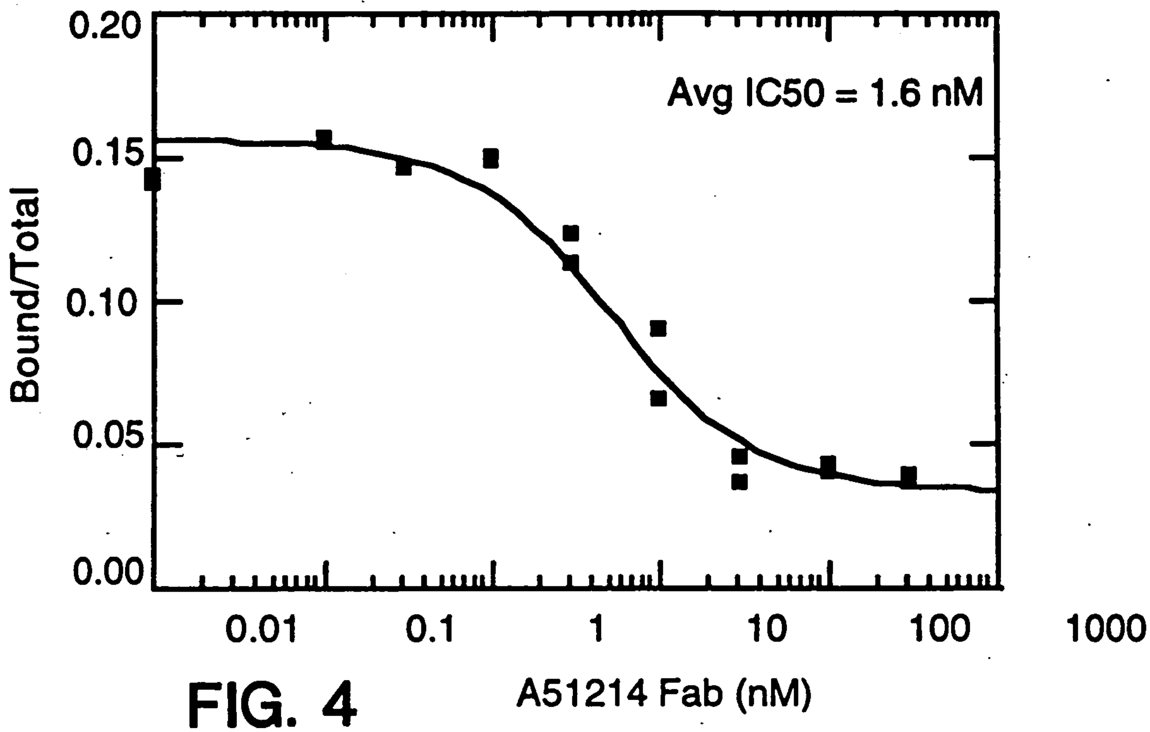

Avg IC50 = 1.6 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
A51214 Fab (nM)

FIG. 4

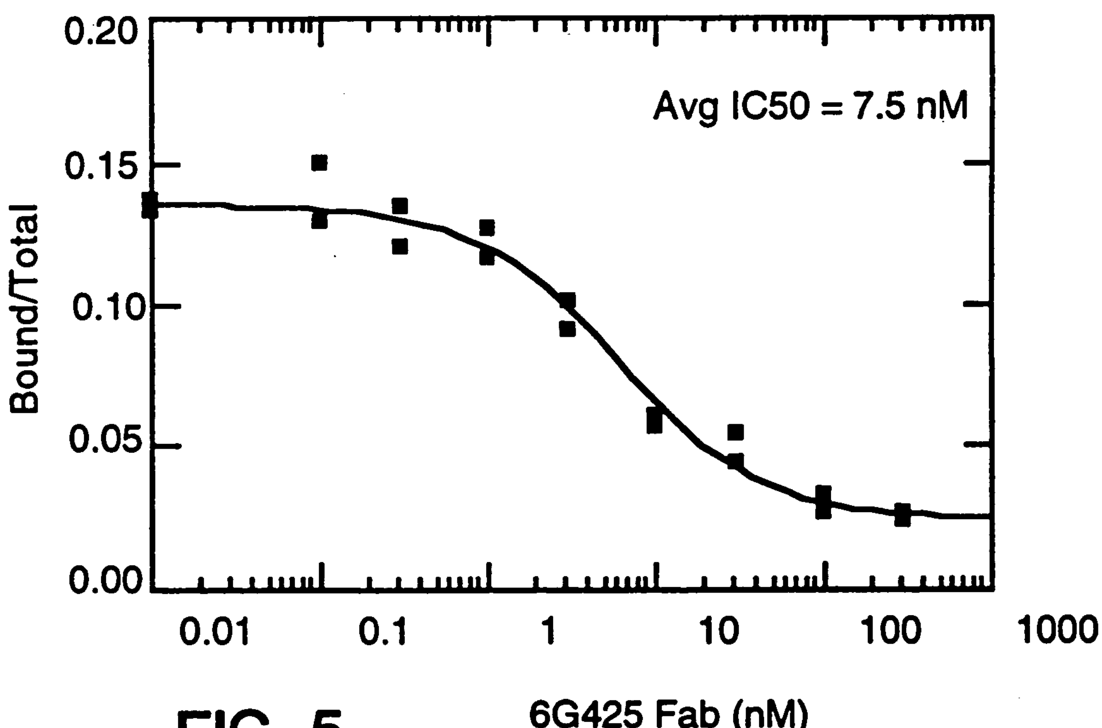

Avg IC50 = 7.5 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
6G425 Fab (nM)

FIG. 5

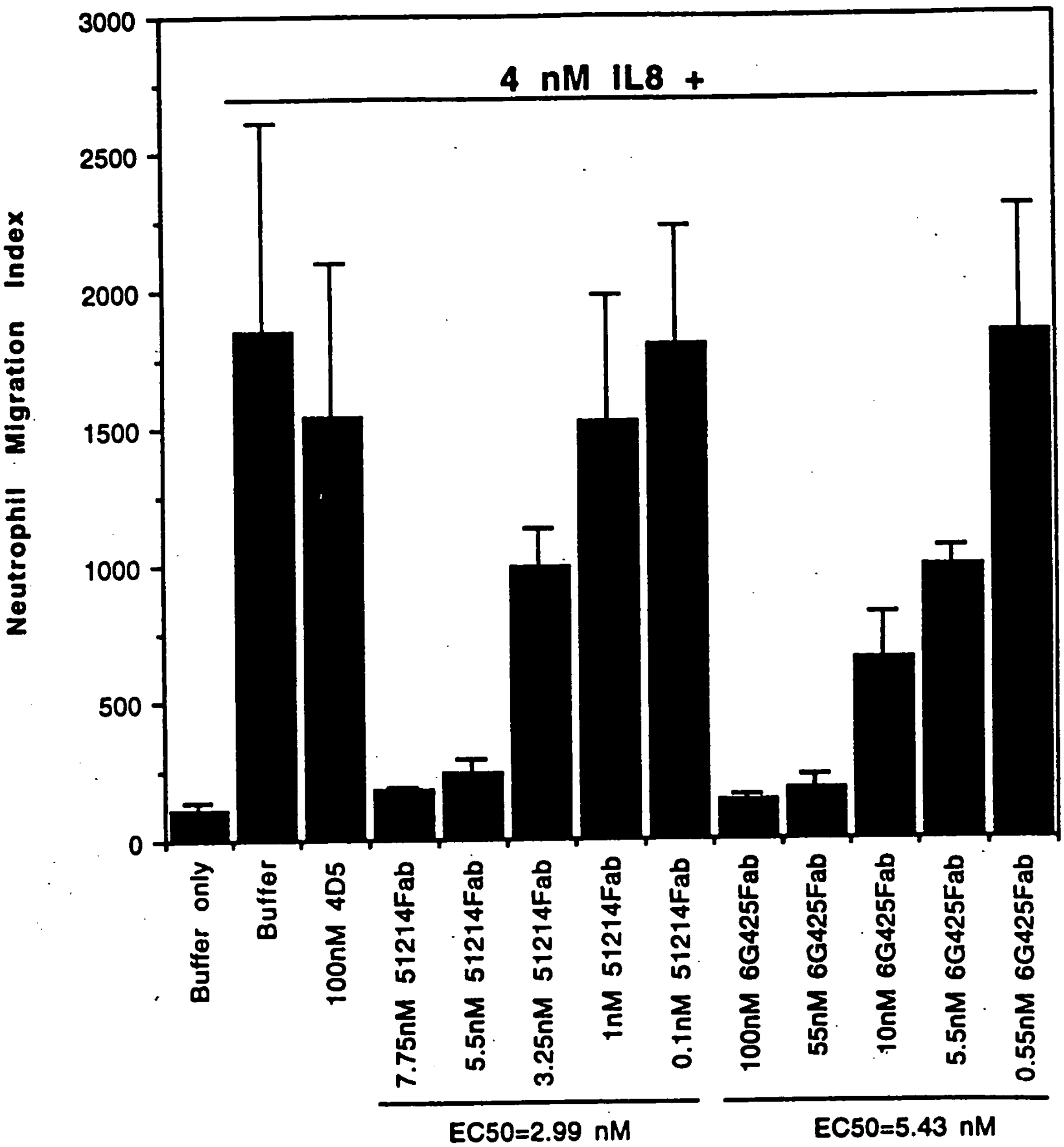

3000
2500
2000
1500
1000
500
0
Neutrophil Migration Index
4 nM IL8 +
Buffer only
Buffer
100nM 4D5
7.75nM 51214Fab
5.5nM 51214Fab
3.25nM 51214Fab
1nM 51214Fab
0.1nM 51214Fab
100nM 6G425Fab
55nM 6G425Fab
10nM 6G425Fab
5.5nM 6G425Fab
0.55nM 6G425Fab
EC50=2.99 nM
EC50=5.43 nM

FIG. 6

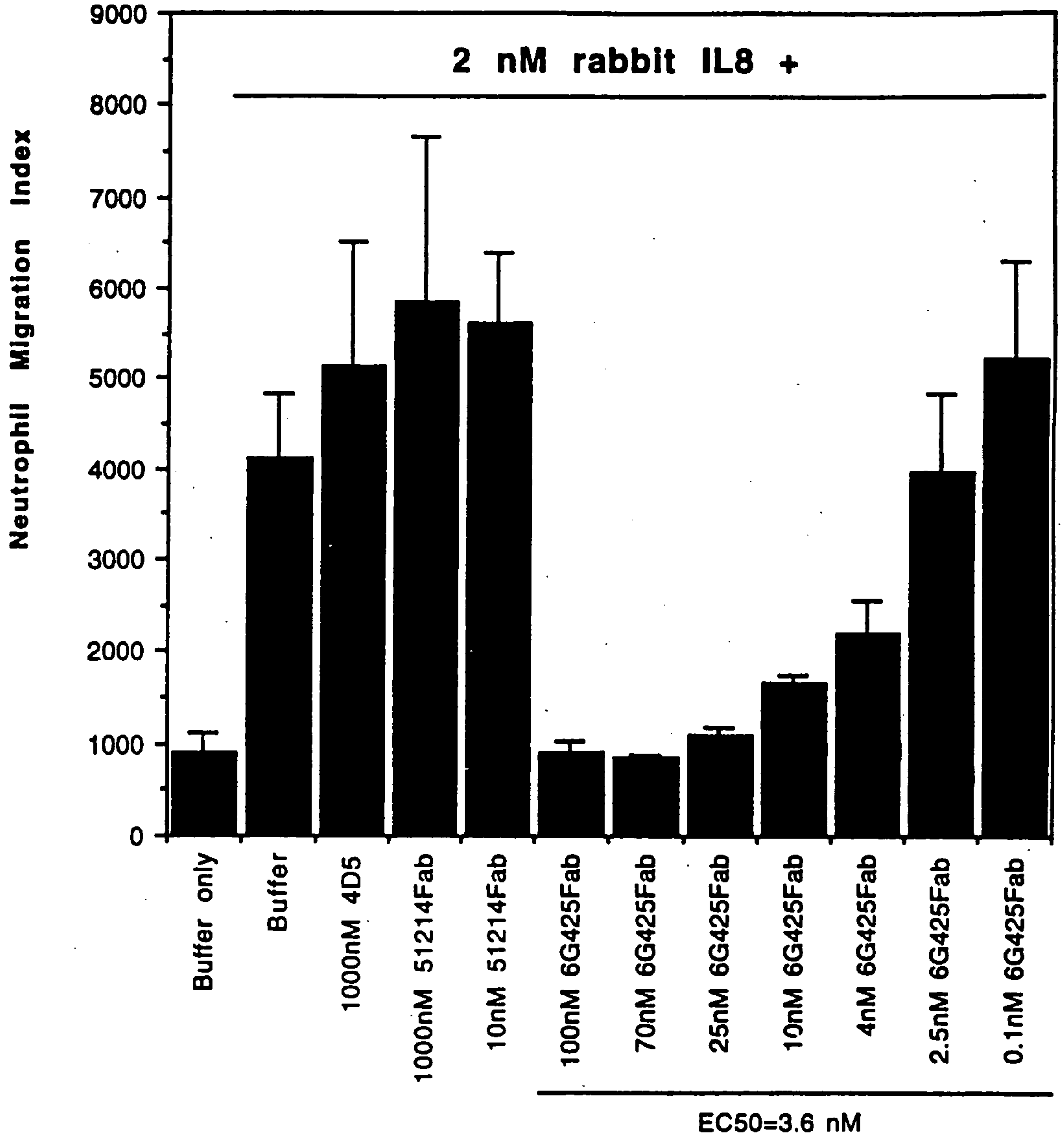

2 nM rabbit IL8 +
Neutrophil Migration Index
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Buffer only
Buffer
1000nM 4D5
1000nM 51214Fab
10nM 51214Fab
100nM 6G425Fab
70nM 6G425Fab
25nM 6G425Fab
10nM 6G425Fab
4nM 6G425Fab
2.5nM 6G425Fab
0.1nM 6G425Fab
EC50=3.6 nM

FIG. 7

FIG. 8

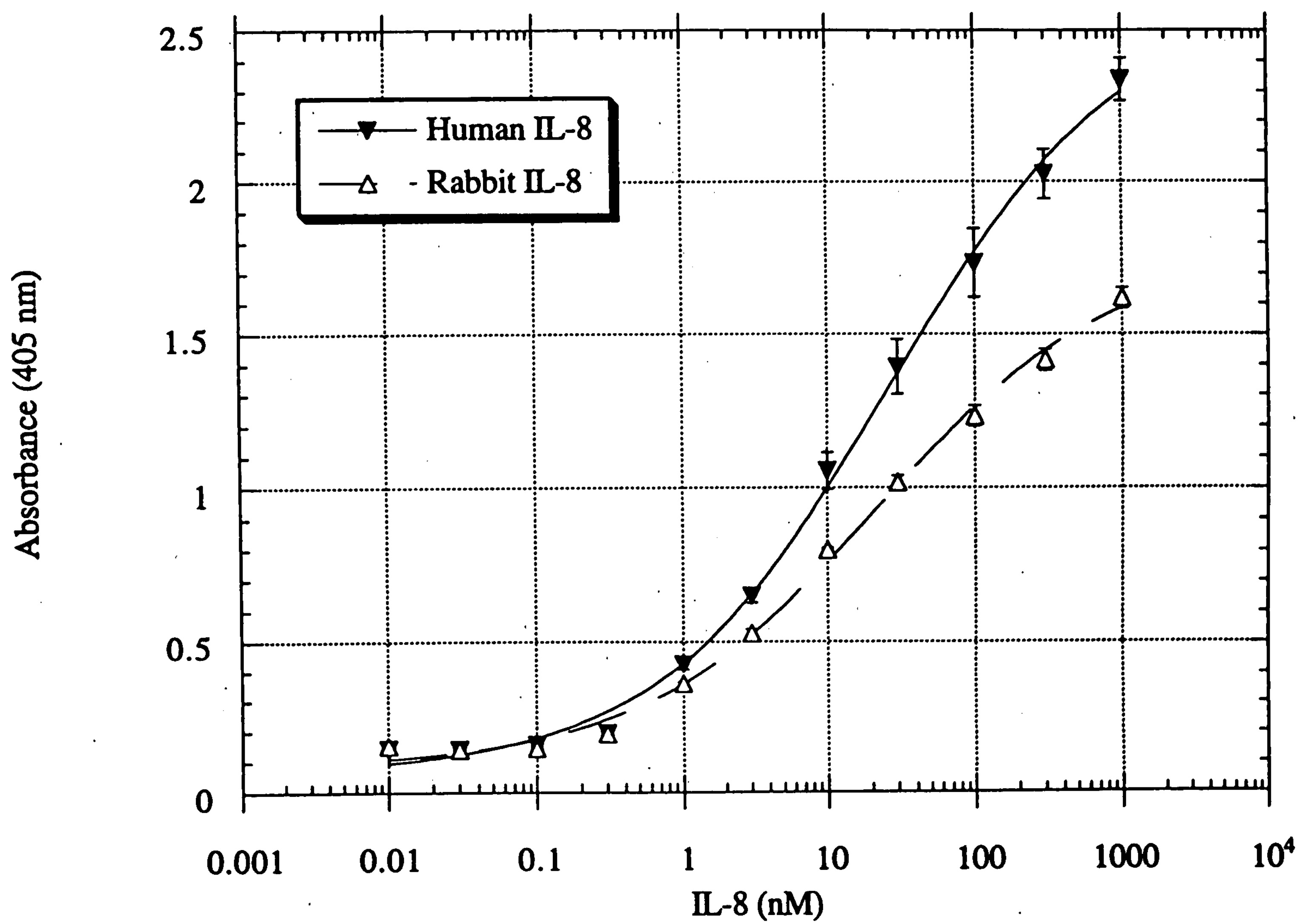

2.5
2
1.5
1
0.5
0
Absorbance (405 nm)
Human IL-8
- Rabbit IL-8
0.001
0.01
0.1
1
10
100
1000
10^4
IL-8 (nM)

FIG. 9

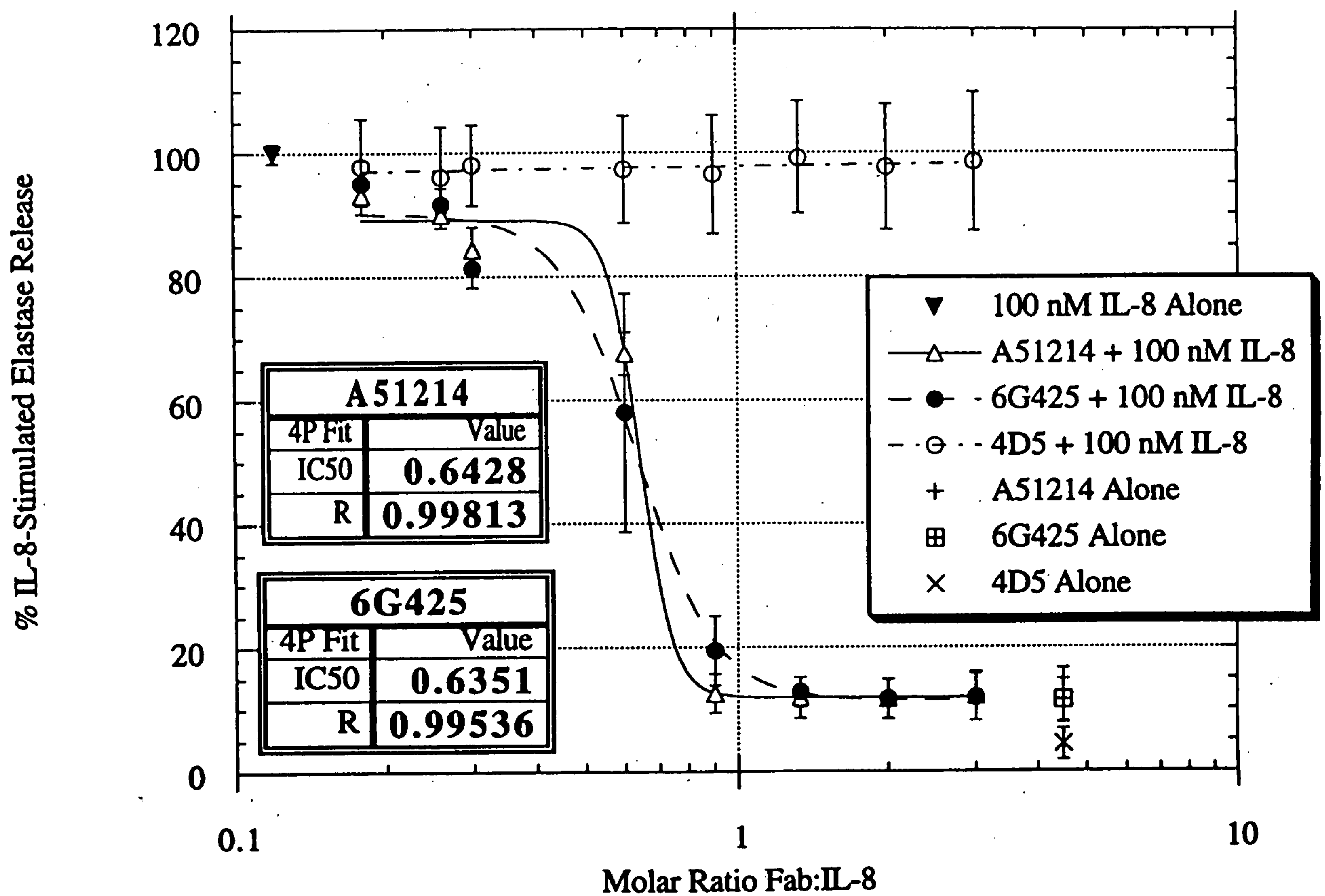
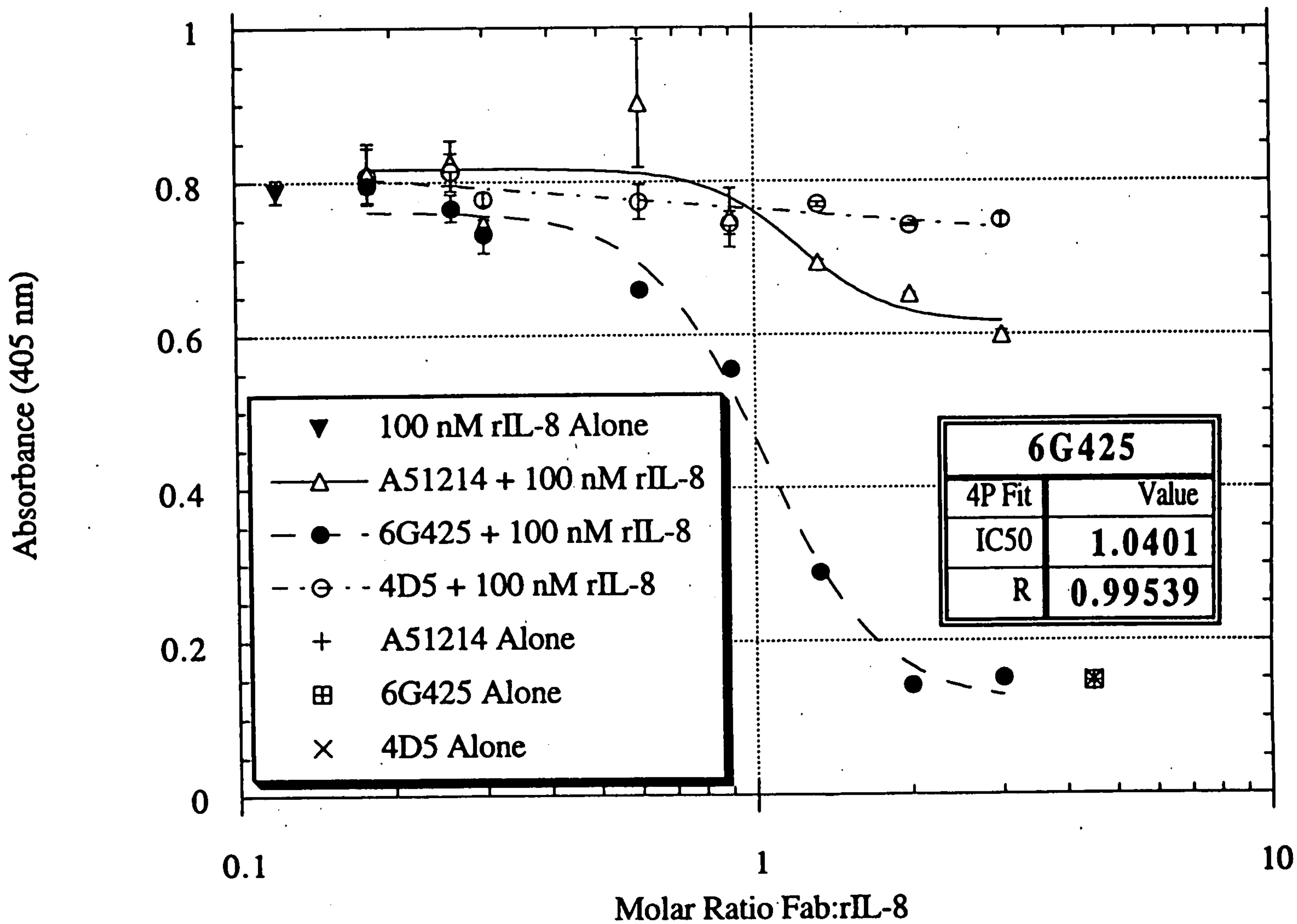

120
100
80
60
40
20
0
% IL-8-Stimulated Elastase Release
0.1
1
10
Molar Ratio Fab:IL-8
100 nM IL-8 Alone
A51214 + 100 nM IL-8
6G425 + 100 nM IL-8
4D5 + 100 nM IL-8
A51214 Alone
6G425 Alone
4D5 Alone
A51214
4P Fit | Value
IC50 | 0.6428
R | 0.99813
6G425
4P Fit | Value
IC50 | 0.6351
R | 0.99536

FIG. 10



Absorbance (405 nm)
1
0.8
0.6
0.4
0.2
0
0.1
1
10
Molar Ratio Fab:rIL-8
100 nM rIL-8 Alone
A51214 + 100 nM rIL-8
6G425 + 100 nM rIL-8
4D5 + 100 nM rIL-8
A51214 Alone
6G425 Alone
4D5 Alone
6G425
4P Fit
Value
IC50
1.0401
R
0.99539

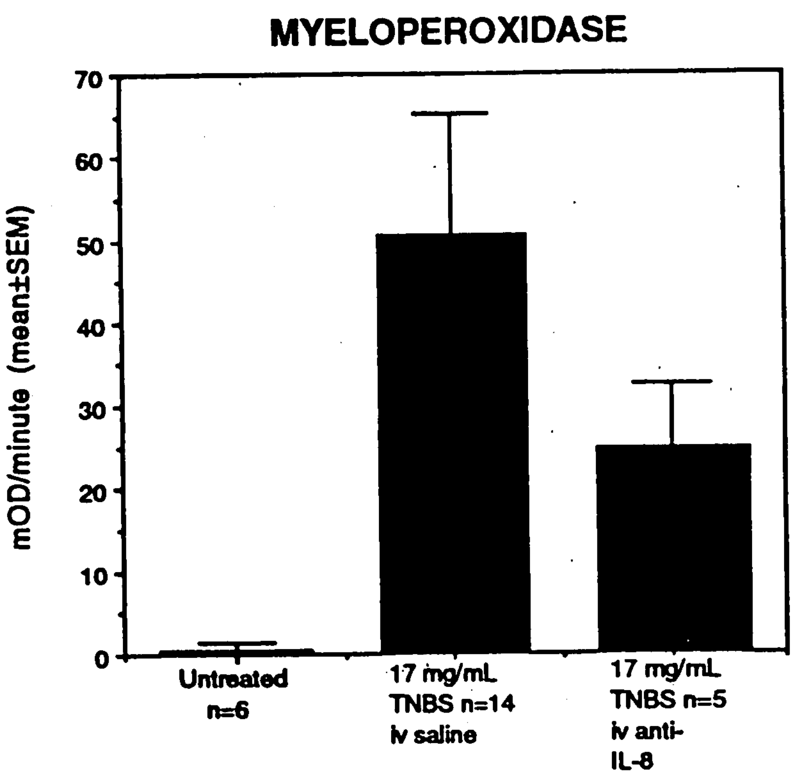


FIG. 11A

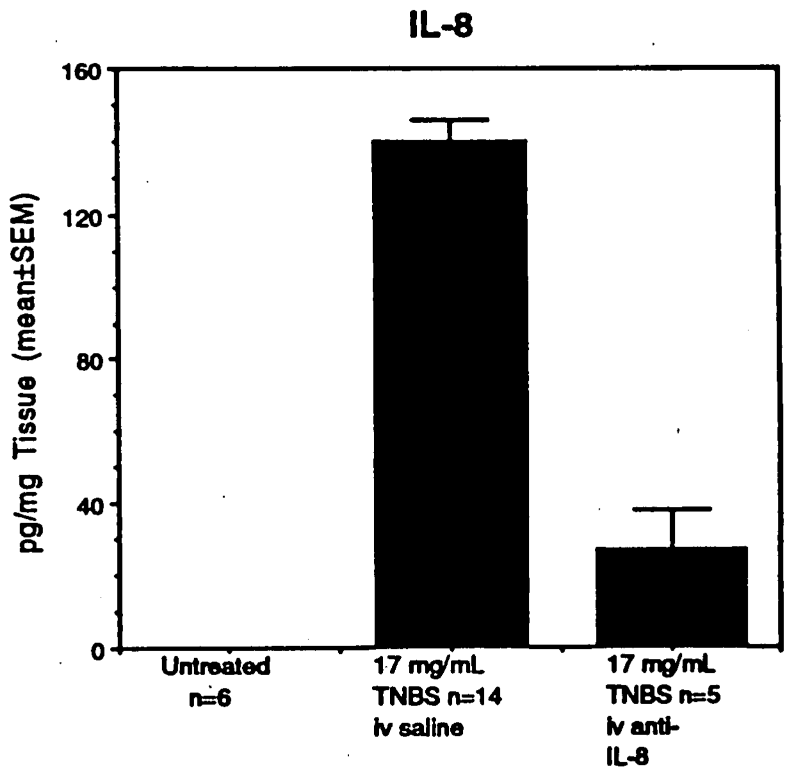


FIG. 11B

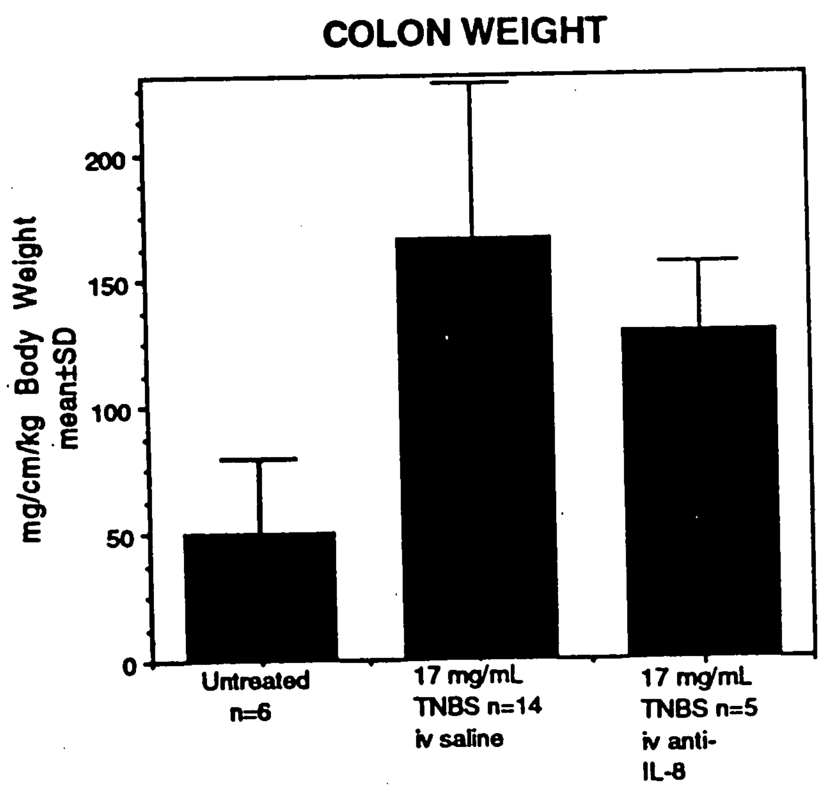


FIG. 11C

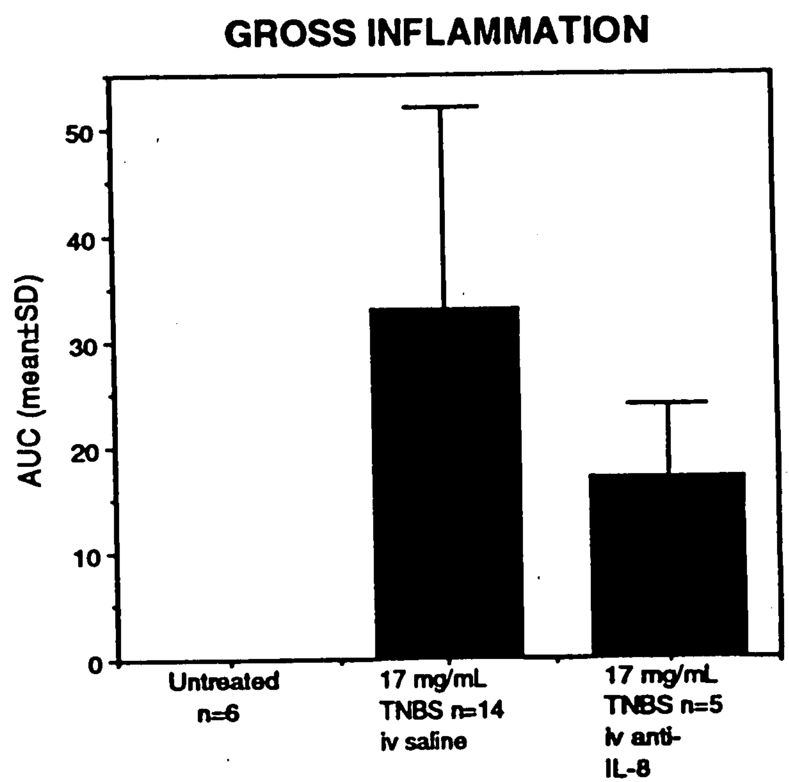


FIG. 11D

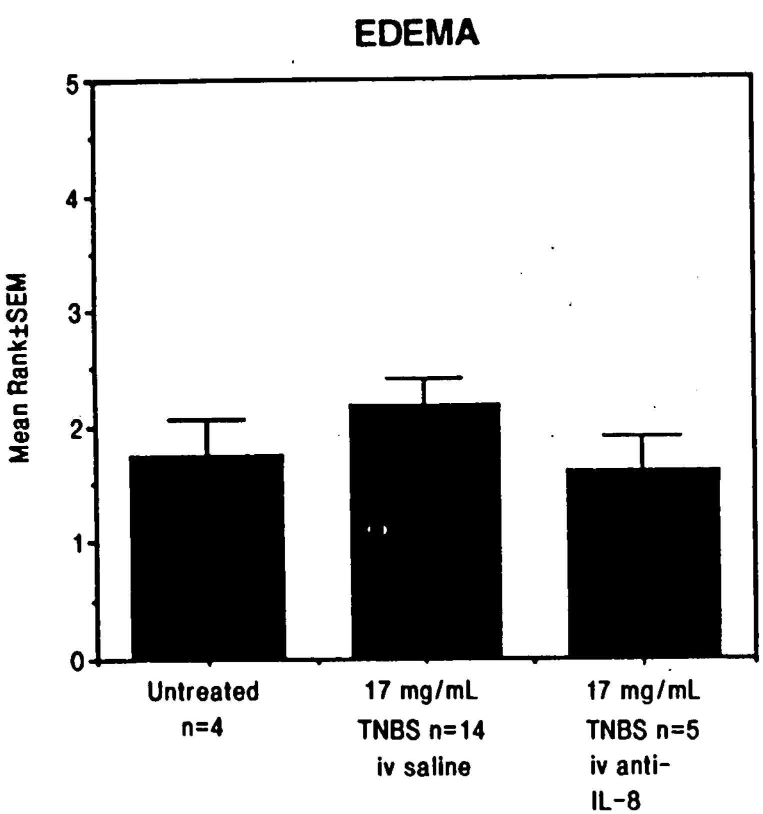


FIG. 11E

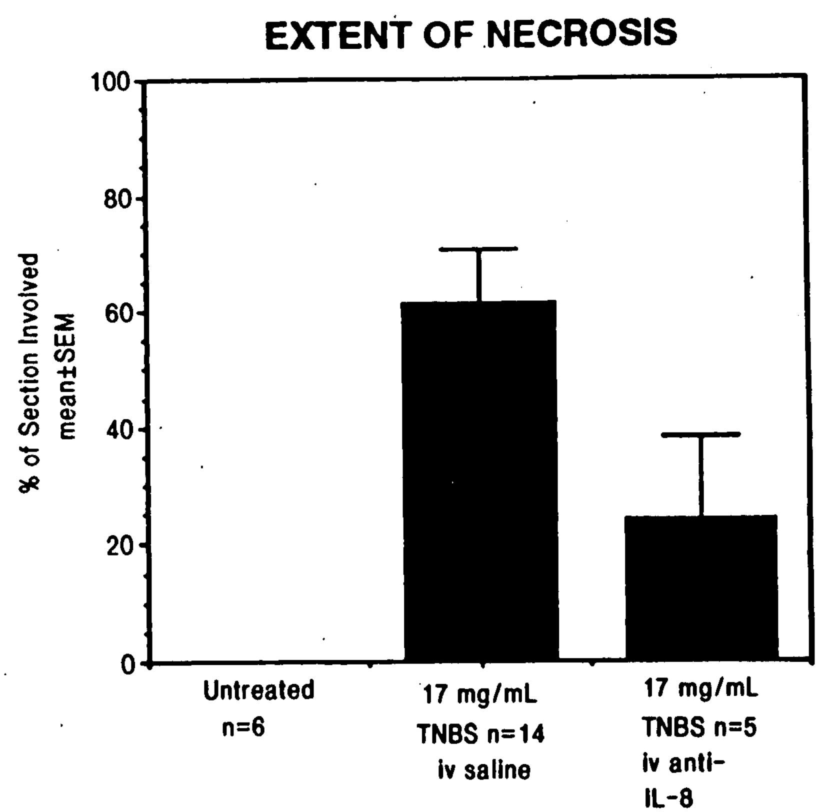


FIG. 11F

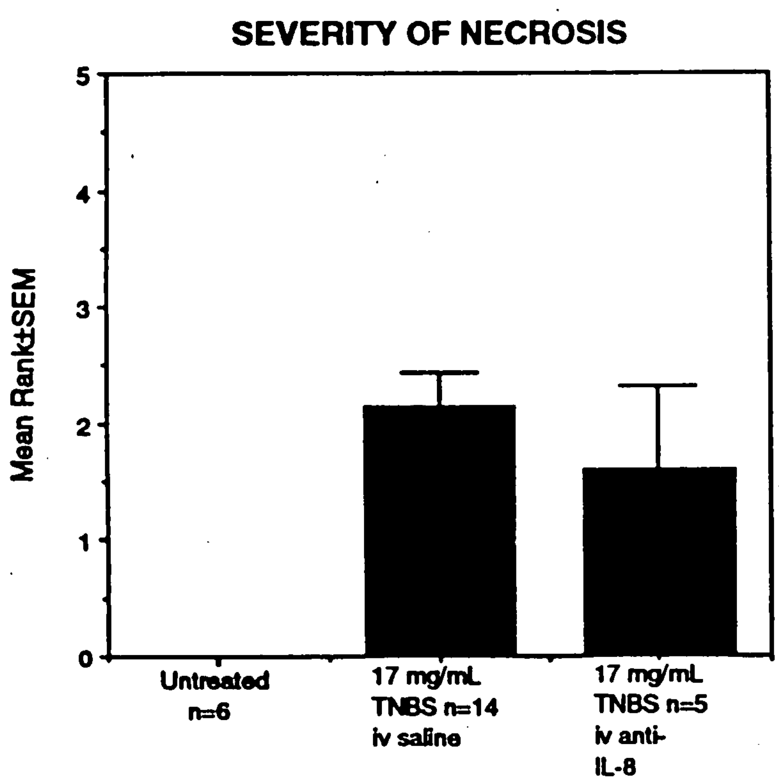


FIG. 11G

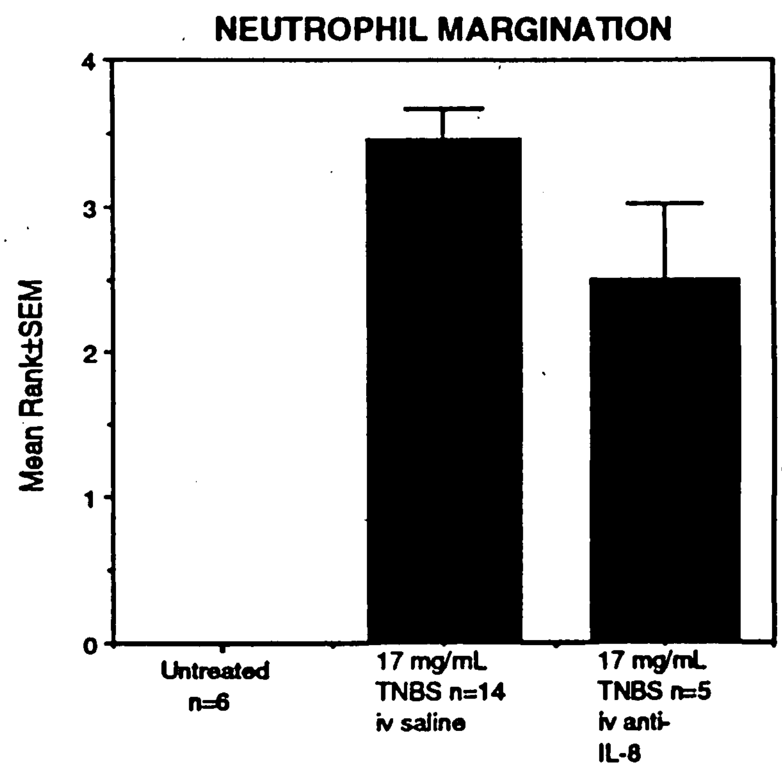


FIG. 11H

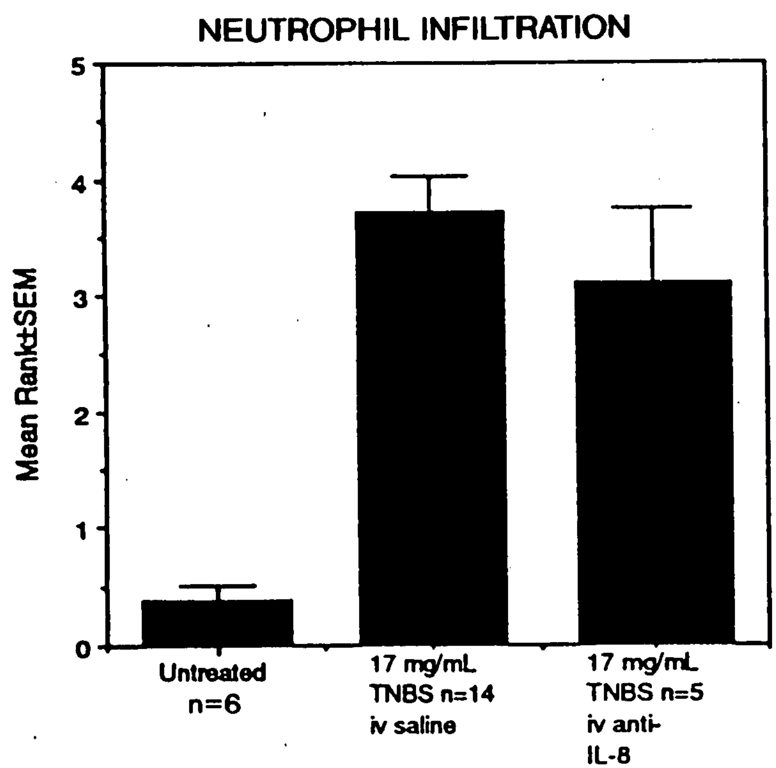


FIG. 11I

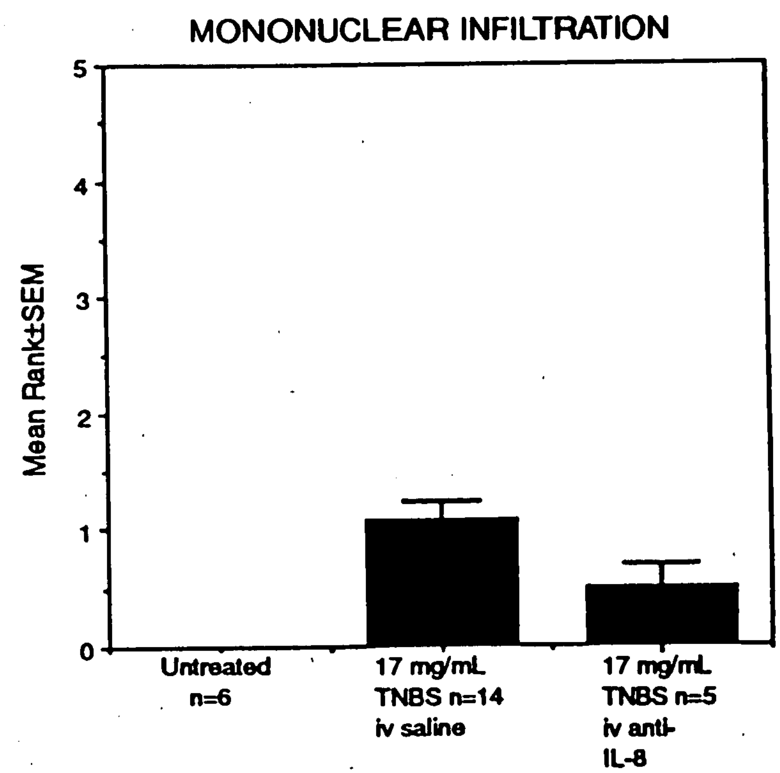
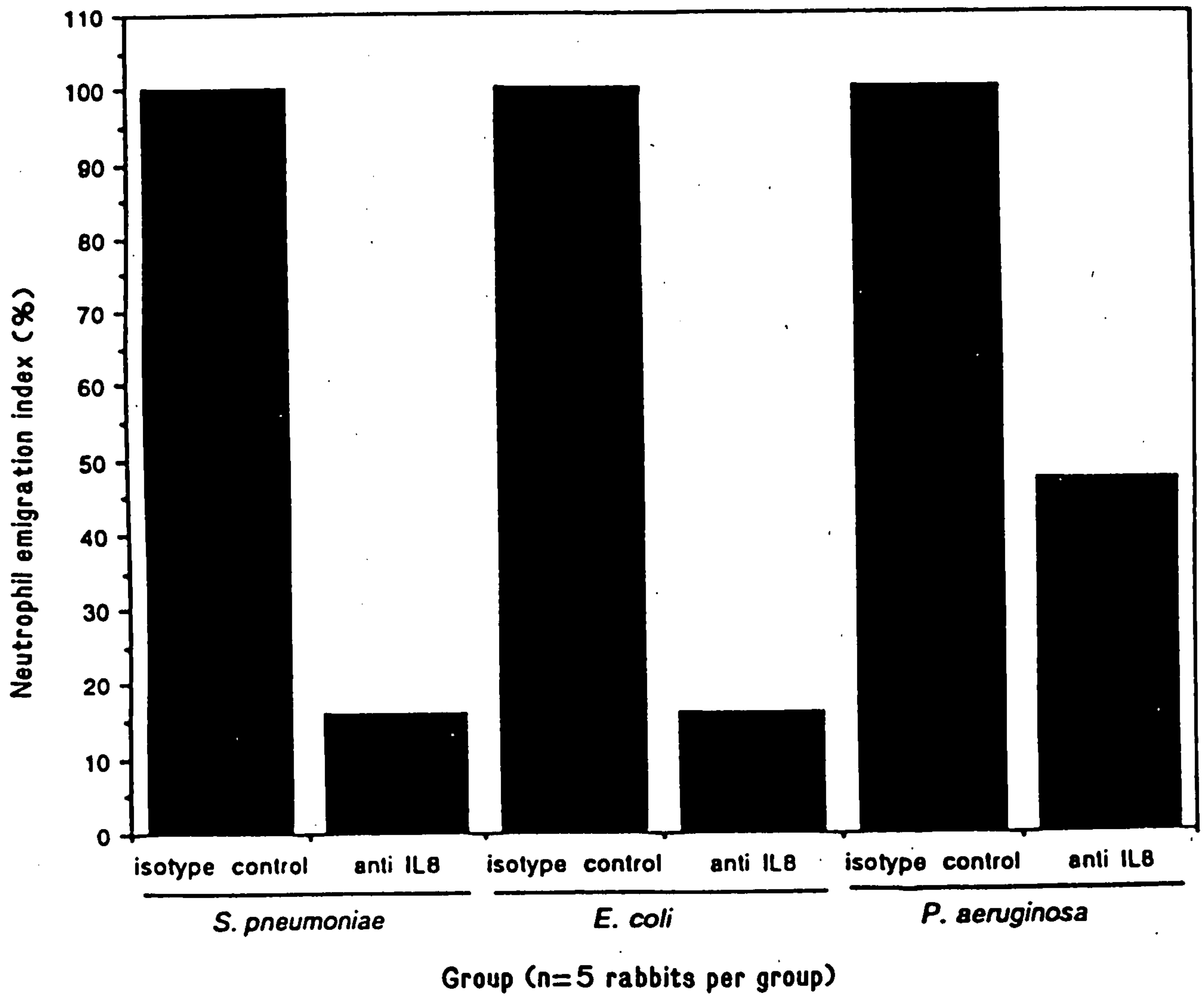


FIG. 11J

FIG. 12



110
100
90
80
70
60
50
40
30
20
10
0
Neutrophil emigration index (%)
isotype control
anti IL8
isotype control
anti IL8
isotype control
anti IL8
S. pneumoniae
E. coli
P. aeruginosa
Group (n=5 rabbits per group)

Light Chain Primers:

FIG. 13

MKLC-1, 22mer

5' CAGTCCAACTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

FIG. 14

Light chain forward primer

SL001A-2 35 mer

```
5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC  3'
                      T  T        T
                                  A
```

Light chain reverse primer

SL001B 37 mer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3'

Heavy chain forward primer

FIG. 15

SL002B 39 mer

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3'
                      T           C
                      G
                      A
```

Heavy chain reverse primer

SL002B 39-MER

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3'
                      T
                      A
                      G
```

```
  1 GACATTGTCA TGACACAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA CAGGGTCAGC
    CTGTAACAGT ACTGTGTCAG AGTTTTTAAG TACAGGTGTA GTCATCCTCT GTCCCAGTCG
  1 D  I  V  M   T  Q  S   Q  K  F   M  S  T  S   V  G  D   R  V  S

 61 GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG CCTGGTATCA ACAGAAACCA
    CAGTGGACGT TCCGGTCAGT CTTACACCCA TGATTACATC GGACCATAGT TGTCTTTGGT
 21 V  T  C  K   A  S  Q   N  V  G   T  N  V  A   W  Y  Q   Q  K  P
             *   *  *  *   *  *  *   *  *  *  *
                               CDR #1

121 GGGCAATCTC CTAAAGCACT GATTTACTCG TCATCCTACC GGTACAGTGG AGTCCCTGAT
    CCCGTTAGAG GATTTCGTGA CTAAATGAGC AGTAGGATGG CCATGTCACC TCAGGGACTA
 41 G  Q  S  P   K  A  L   I  Y  S   S  S  Y  R   Y  S  G   V  P  D
                                *   *  *  *  *   *  *
                                        CDR #2

181 CGCTTCACAG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCCA TGTGCAGTCT
    GCGAAGTGTC CGTCACCTAG ACCCTGTCTA AAGTGAGAGT GGTAGTCGGT ACACGTCAGA
 61 R  F  T  G   S  G  S   G  T  D   F  T  L  T   I  S  H   V  Q  S

241 GAAGACTTGG CAGACTATTT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT
    CTTCTGAACC GTCTGATAAA GACAGTCGTT ATATTGTAGA TAGGAGAGTG CAAGCCAGGA
 81 E  D  L  A   D  Y  F   C  Q  Q   Y  N  I  Y   P  L  T   F  G  P
                              *  *   *  *  *  *   *  *  *
                                 CDR #3

301 GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC CATCTTCCCA
    CCCTGGTTCG ACCTCAACTT TGCCCGACTA CGACGTGGTG GTTGACATAG GTAGAAGGGT
101 G  T  K  L   E  L  K   R  A  D   A  A  P  P   T  V  S   I  F  P

      BstBI
361 CCATTCGAA
    GGTAAGCTT
121 P  F  E
```

FIG. 16

```
  1 TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG GAGGCTTAGT
    AAGATAACGA TGTTTGCGCA TGCGACTCCA CGTCGACCAC CTCAGACCCC CTCCGAATCA
  1                               E  V    Q  L  V    E  S  G  G    G  L  V

 61 GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT GGATTCATAT TCAGTAGTTA
    CGGCGGACCT CCCAGGGACT TTGAGAGGAC ACGTCGGAGA CCTAAGTATA AGTCATCAAT
 13  P  P  G    G  S  L  K    L  S  C    A  A  S    G  F  I  F    S  S  Y
                                                                     *  *
                                                         CDR #1

121 TGGCATGTCT TGGGTTCGCC AGACTCCAGG CAAGAGCCTG GAGTTGGTCG CAACCATTAA
    ACCGTACAGA ACCCAAGCGG TCTGAGGTCC GTTCTCGGAC CTCAACCAGC GTTGGTAATT
 33  G  M  S    W  V  R  Q    T  P  G    K  S  L    E  L  V  A    T  I  N
     *  *  *                                                  *  *  *

181 TAATAATGGT GATAGCACCT ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG
    ATTATTACCA CTATCGTGGA TAATAGGTCT GTCACACTTC CCGGCTAAGT GGTAGAGGGC
 53  N  N  G    D  S  T  Y    Y  P  D    S  V  K    G  R  F  T    I  S  R
     *  *  *    *  *  *  *    *  *  *    *  *  *
                   CDR #2

241 AGACAATGCC AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC
    TCTGTTACGG TTCTTGTGGG ACATGGACGT TTACTCGTCA GACTTCAGAC TCCTGTGTCG
 73  D  N  A    K  N  T  L    Y  L  Q    M  S  S    L  K  S  E    D  T  A

301 CATGTTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT ACTGGGGCCA
    GTACAAAATG ACACGTTCTC GGGAGTAATC AAGCCGATGA ACCAAACCAA TGACCCCGGT
 93  M  F  Y    C  A  R  A    L  I  S    S  A  T    W  F  G  Y    W  G  Q
                          *    *  *  *    *  *  *    *  *  *  *
                                     CDR #3

361 AGGGACTCTG GTCACTGTCT CTGCAGCCAA AACAACAGCC CCATCTGTCT
    TCCCTGAGAC CAGTGACAGA GACGTCGGTT TTGTTGTCGG GGTAGACAGA
113  G  T  L    V  T  V  S    A  A  K    T  T  A    P  S  V  Y

         ApaI
411   ATCCGGG
      TAGGCCC
130     P
```

FIG. 17

FIG. 18

VL.front 31-MER

5' ACAAACGCGTACGCTGATATCGTCATGACAG 3'

VL.rear 31-MER

5' GCAGCATCAGCTCTTCGAAGCTCCAGCTTGG 3'

VH.front.SPE 21-MER

5' CCACTAGTACGCAAGTTCACG 3'

VH.rear 33-MER

5' GATGGGCCCTTGGTGGAGGCTGCAGAGACAGTG 3'

```
  1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCGTACGCTG ATATCGTCAT GACACAGTCT CAAAAATTCA TGTCCACATC AGTAGGAGAC
    CGCATGCGAC TATAGCAGTA CTGTGTCAGA GTTTTTAAGT ACAGGTGTAG TCATCCTCTG
 -3 A  Y  A  D   I  V  M   T  Q  S   Q  K  F  M   S  T  S   V  G  D

121 AGGGTCAGCG TCACCTGCAA GGCCAGTCAG AATGTGGGTA CTAATGTAGC CTGGTATCAA
    TCCCAGTCGC AGTGGACGTT CCGGTCAGTC TTACACCCAT GATTACATCG GACCATAGTT
 18 R  V  S  V   T  C  K   A  S  Q   N  V  G  T   N  V  A   W  Y  Q
                         *   *  *  *   *  *  *  *   *  *  *
                                       CDR #1

181 CAGAAACCAG GGCAATCTCC TAAAGCACTG ATTTACTCGT CATCCTACCG GTACAGTGGA
    GTCTTTGGTC CCGTTAGAGG ATTTCGTGAC TAAATGAGCA GTAGGATGGC CATGTCACCT
 38 Q  K  P  G   Q  S  P   K  A  L   I  Y  S  S   S  Y  R   Y  S  G
                                             *  *   *  *  *   *  *
                                                    CDR #2

241 GTCCCTGATC GCTTCACAGG CAGTGGATCT GGGACAGATT TCACTCTCAC CATCAGCCAT
    CAGGGACTAG CGAAGTGTCC GTCACCTAGA CCCTGTCTAA AGTGAGAGTG GTAGTCGGTA
 58 V  P  D  R   F  T  G   S  G  S   G  T  D  F   T  L  T   I  S  H

301 GTGCAGTCTG AAGACTTGGC AGACTATTTC TGTCAGCAAT ATAACATCTA TCCTCTCACG
    CACGTCAGAC TTCTGAACCG TCTGATAAAG ACAGTCGTTA TATTGTAGAT AGGAGAGTGC
 78 V  Q  S  E   D  L  A   D  Y  F   C  Q  Q  Y   N  I  Y   P  L  T
                                        *  *  *   *  *  *   *  *  *
                                                     CDR #3

                               BstBI
361 TTCGGTCCTG GGACCAAGCT GGAGCTTCGA AGAGCTGTGG CTGCACCATC TGTCTTCATC
    AAGCCAGGAC CCTGGTTCGA CCTCGAAGCT TCTCGACACC GACGTGGTAG ACAGAAGTAG
 98 F  G  P  G   T  K  L   E  L  R   R  A  V  A   A  P  S   V  F  I

421 TTCCCGCCAT CTGATGAGCA GTTGAAATCT GGAACTGCTT CTGTTGTGTG CCTGCTGAAT
    AAGGGCGGTA GACTACTCGT CAACTTTAGA CCTTGACGAA GACAACACAC GGACGACTTA
118 F  P  P  S   D  E  Q   L  K  S   G  T  A  S   V  V  C   L  L  N

481 AACTTCTATC CCAGAGAGGC CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT
    TTGAAGATAG GGTCTCTCCG GTTTCATGTC ACCTTCCACC TATTGCGGGA GGTTAGCCCA
138 N  F  Y  P   R  E  A   K  V  Q   W  K  V  D   N  A  L   Q  S  G

541 AACTCCCAGG AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC
    TTGAGGGTCC TCTCACAGTG TCTCGTCCTG TCGTTCCTGT CGTGGATGTC GGAGTCGTCG
158 N  S  Q  E   S  V  T   E  Q  D   S  K  D  S   T  Y  S   L  S  S

601 ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG CGAAGTCACC
    TGGGACTGCG ACTCGTTTCG TCTGATGCTC TTTGTGTTTC AGATGCGGAC GCTTCAGTGG
178 T  L  T  L   S  K  A   D  Y  E   K  H  K  V   Y  A  C   E  V  T

661 CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA GGGGAGAGTG
    GTAGTCCCGG ACTCGAGCGG GCAGTGTTTC TCGAAGTTGT CCCCTCTCAC
198 H  Q  G  L   S  S  P   V  T  K   S  F  N  R   G  E  C

711   TTAA
      AATT
216   O
```

FIG. 19

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT TAGTGCCGCC TGGAGGGTCC
    CGCATGCGAC TCCACGTCGA CCACCTCAGA CCCCCTCCGA ATCACGGCGG ACCTCCCAGG
 -3 A  Y  A  E   V  Q  L   V  E  S   G  G  G  L   V  P  P   G  G  S

121 CTGAAACTCT CCTGTGCAGC CTCTGGATTC ATATTCAGTA GTTATGGCAT GTCTTGGGTT
    GACTTTGAGA GGACACGTCG GAGACCTAAG TATAAGTCAT CAATACCGTA CAGAACCCAA
 18 L  K  L  S   C  A  A   S  G  F   I  F  S  S   Y  G  M   S  W  V
                                               *   *  *  *   *
                                          CDR #1

181 CGCCAGACTC CAGGCAAGAG CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC
    GCGGTCTGAG GTCCGTTCTC GGACCTCAAC CAGCGTTGGT AATTATTATT ACCACTATCG
 38 R  Q  T  P   G  K  S   L  E  L   V  A  T  I   N  N  N   G  D  S
                                          *  *   *  *  *   *  *  *

241 ACCTATTATC CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC
    TGGATAATAG GTCTGTCACA CTTCCCGGCT AAGTGGTAGA GGGCTCTGTT ACGGTTCTTG
 58 T  Y  Y  P   D  S  V   K  G  R   F  T  I  S   R  D  N   A  K  N
    *  *  *  *   *  *  *   *
      CDR #2

301 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT TTACTGTGCA
    TGGGACATGG ACGTTTACTC GTCAGACTTC AGACTCCTGT GTCGGTACAA AATGACACGT
 78 T  L  Y  L   Q  M  S   S  L  K   S  E  D  T   A  M  F   Y  C  A

361 AGAGCCCTCA TTAGTTCGGC TACTTGGTTT GGTTACTGGG GCCAAGGGAC TCTGGTCACT
    TCTCGGGAGT AATCAAGCCG ATGAACCAAA CCAATGACCC CGGTTCCCTG AGACCAGTGA
 98 R  A  L  I   S  S  A   T  W  F   G  Y  W  G   Q  G  T   L  V  T
       *  *  *   *  *  *   *  *  *   *  *
                   CDR #3
                          ApaI
421 GTCTCTGCAG CCTCCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCTC CTCCAAGAGC
    CAGAGACGTC GGAGGTGGTT CCCGGGTAGC CAGAAGGGGG ACCGTGGGAG GAGGTTCTCG
118 V  S  A  A   S  T  K   G  P  S   V  F  P  L   A  P  S   S  K  S

481 ACCTCTGGGG GCACAGCGGC CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG
    TGGAGACCCC CGTGTCGCCG GGACCCGACG GACCAGTTCC TGATGAAGGG GCTTGGCCAC
138 T  S  G  G   T  A  A   L  G  C   L  V  K  D   Y  F  P   E  P  V

541 ACGGTGTCGT GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA
    TGCCACAGCA CCTTGAGTCC GCGGGACTGG TCGCCGCACG TGTGGAAGGG CCGACAGGAT
158 T  V  S  W   N  S  G   A  L  T   S  G  V  H   T  F  P   A  V  L

601 CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG CAGCTTGGGC
    GTCAGGAGTC CTGAGATGAG GGAGTCGTCG CACCACTGGC ACGGGAGGTC GTCGAACCCG
178 Q  S  S  G   L  Y  S   L  S  S   V  V  T  V   P  S  S   S  L  G
```

FIG. 20A

```
661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA
    TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT TGTGGTTCCA CCTGTTCTTT
198  T  Q  T  Y   I  C  N   V  N  H   K  P  S  N   T  K  V   D  K  K

721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGA
    CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT
218  V  E  P  K   S  C  D   K  T  H   T  O
```

FIG. 20B

Light Chain Primers:

MKLC-1, 22mer

5' CAGTCCAACTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

FIG. 21

Light chain forward primer

6G4.light.Nsi 36-MER

```
5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC  3'
                    T   T           T       T
                                    A       A
```

Light chain reverse primer

6G4.light.Mun 35-MER

5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3'

FIG. 22

Heavy chain forward primer

6G4.heavy.Mlu 32-MER

```
5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG  3'
                          T       C
```

Heavy chain reverse primer

SL002B 39-MER

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC  3'
                      T
                      A
                      G
```

FIG. 23

```
 70 G ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT
    C TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
  1 D  I  V  M  T  Q  T  P  L  S  L  P  V  S  L  G  D

121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
    GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
 18 Q  A  S  I  S  C  R  S  S  Q  S  L  V  H  G  I  G  N  T  Y
                      *  *  *  *  *  *  *  *  *  *  *  *  *  *
                                  CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
    AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
 38 L  H  W  Y  L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S
    *  *                                                 *  *  *
                                                         CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA
    TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T
    *  *  *  *

301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
    GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
 78 L  R  I  S  R  V  E  A  E  D  L  G  L  Y  F  C  S  Q  S  T
                                                      *  *  *  *
                                                      CDR #3

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGATGCTGCA
    GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACTACGACGT
 98 H  V  P  L  T  F  G  A  G  T  K  L  E  L  K  R  A  D  A  A
    *  *  *  *  *

                                         MunI
421 CCAACTGTAT CCATCTTCCC ACCATCCAGT GAGCAATTGA
    GGTTGACATA GGTAGAAGGG TGGTAGGTCA CTCGTTAACT
118 P  T  V  S  I  F  P  P  S  S  E  Q  L  K
```

FIG. 24

```
 70 G AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
    C TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
  1 E   I  Q  L   Q  Q  S   G  P  E  L   M  K  P   G  A  S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTCAGTA GCCACTACAT GCACTGGGTG
    CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V  K  I  S   C  K  A  S  G  Y   S  F  S  S   H  Y  M   H  W  V
                                                 *   *   *   *    *
                                                CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
    TTCGTCTCGG TACCTTTCTC GGAACTCACC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K  Q  S  H   G  K  S   L  E  W   I  G  Y  I   D  P  S   N  G  E
                                           *  *   *  *  *   *  *  *
                                                      CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
    TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAACT GACATCTGTG TAGAAGGTCG
 58 T  T  Y  N   Q  K  F   K  G  K   A  T  L  T   V  D  T   S  S  S
    *  *  *  *   *  *  *   *  *

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
    TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T  A  N  V   H  L  S   S  L  T   S  D  D  S   A  V  Y   F  C  A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
    TCTCCCCTGA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R  G  D  Y   R  Y  N   G  D  W   F  F  D  V   W  G  A   G  T  T
       *  *  *   *  *  *   *  *  *   *  *  *  *
                    CDR #3
     BstEII                                        ApaI
421 GTCACCGTCT CCTCCGCCAA AACCGACAGC CCCATCGGTC TATCCGGGCC
    CAGTGGCAGA GGAGGCGGAT TTGGCTGTCG GGGTAGCCAG ATAGGCCCGG
118 V  T  V  S   S  A  K   T  D  S   P  I  G  L   S  G  P

471 CATC
    GTAG
135  I
```

FIG. 25

5' CTTGGTGGAGGCGGAGGAGACG 3'

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATTCATCTTCC 3'

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3'

SYN.Apa 22 MER

5' CTTGGTGGAGGCGGAGGAGACG 3'

FIG. 26

```
  1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAT
    TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTA
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT
    CGTATGCGAC TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
 -3 A  Y  A  D   I  V  M   T  Q  T   P  L  S  L   P  V  S   L  G  D

121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
    GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
 18 Q  A  S  I   S  C  R   S  S  Q   S  L  V  H   G  I  G   N  T  Y
                        *   *  *  *   *  *  *  *   *  *  *   *  *  *
                                           CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
    AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
 38 L  H  W  Y   L  Q  K   P  G  Q   S  P  K  L   L  I  Y   K  V  S
    *  *                                                    *  *  *
                                                           CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA
    TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N  R  F  S   G  V  P   D  R  F   S  G  S  G   S  G  T   D  F  T
    *  *  *  *

301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
    GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
 78 L  R  I  S   R  V  E   A  E  D   L  G  L  Y   F  C  S   Q  S  T
                                                     *   *  *  *
                                                   CDR #3

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGTTGCTGCA
    GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACAACGACGT
 98 H  V  P  L   T  F  G   A  G  T   K  L  E  L   K  R  A   V  A  A
    *  *  *  *   *

421 CCAACTGTAT TCATCTTCCC ACCATCCAGT GAGCAATTGA AATCTGGAAC TGCCTCTGTT
    GGTTGACATA AGTAGAAGGG TGGTAGGTCA CTCGTTAACT TTAGACCTTG ACGGAGACAA
118 P  T  V  F   I  F  P   P  S  S   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y
```

FIG. 27A

```
661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G

721 GAGTGTTAA
    CTCACAATT
218 E  C  O
```

FIG. 27B

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N  I  A  F  L  L  A  S  M  F  V  F  S  I  A  T  N

 61 GCGTACGCTG AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
    CGCATGCGAC TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
 -3 A  Y  A  E  I  Q  L  Q  Q  S  G  P  E  L  M  K  P  G  A  S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTCAGTA GCCACTACAT GCACTGGGTG
    CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V  K  I  S  C  K  A  S  G  Y  S  F  S  S  H  Y  M  H  W  V
                                            *  *  *  *  *
                                       CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
    TTCGTCTCGG TACCTTTCTC GGAACTCACC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K  Q  S  H  G  K  S  L  E  W  I  G  Y  I  D  P  S  N  G  E
                                      *  *  *  *  *  *  *  *
                                               CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
    TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAACT GACATCTGTG TAGAAGGTCG
 58 T  T  Y  N  Q  K  F  K  G  K  A  T  L  T  V  D  T  S  S  S
    *  *  *  *  *  *  *  *  *

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
    TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T  A  N  V  H  L  S  S  L  T  S  D  D  S  A  V  Y  F  C  A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
    TCTCCCCTGA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R  G  D  Y  R  Y  N  G  D  W  F  F  D  V  W  G  A  G  T  T
       *  *  *  *  *  *  *  *  *  *  *  *  *
             CDR #3

421 GTCACCGTCT CCTCCGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
    CAGTGGCAGA GGAGGCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG
118 V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S

481 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA
    TTCTCGTGGA GACCCCCGTG TCGCCGGGAC CCGACGGACC AGTTCCTGAT GAAGGGGCTT
138 K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E

541 CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT
    GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA
158 P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A

601 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC
    CAGGATGTCA GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGGCACGG GAGGTCGTCG
178 V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S
```

FIG. 28A

```
661 TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC
    AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG GTTCCACCTG
198 L  G  T  Q   T  Y  I   C  N  V   N  H  K  P   S  N  T   K  V  D

721 AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GA
    TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CT
218 K  K  V  E   P  K  S   C  D  K   T  H  T  O
```

FIG. 28B

Variable Light Chain Domain

```
                  10        20       abcde 30        40
6G425     DIVMTQTPLSLPVSLGDQASISCRSSQSLVHGIGNTYLHWYLQKPGQSPKLLIY
            #   # #  ## #  ### #                   #    ##
F(ab)-1   DIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTYLHWYQQKPGKAPKLLIY
                                  # ##########
humκI     DIQMTQSPSSLSASVGDRVTITCRASKTI-----SKYLAWYQQKPGKAPKLLIY
                                   ============
                                 ++++++++++++++++
                                        L1

          50        60        70        80        90       100
6G425     YKVSNRFSGVPDRFSDSGSGTDFTLRISRVEAEDLGLYFCSQSTHVPLTFGAGTKLELKR
                     #   #         #  ##### ### #            #   # #
F(ab)-1   YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPLTFGQGTKVEIKR
           ## ###                                 # ####
humκI     YSGSTLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEYPLTFGQGTKVEIKR
           ===                                      ======
           +++++++                                +++++++++
               L2                                    L3
```

Variable Heavy Chain Domain

```
                  10        20        30        40
6G425     EIQLQQSGPELMKPGASVKISCKASGYSFSSHYMHWVKQSHGKSLEWI
           #  ##  ## ##  # ###   #             # ##  #   #
F(ab)-1   EVQLVESGGGLVQPGGSLRLSCAASGYSFSSHYMHWVRQAPGKGLEWV
                                    #  ## # #
humIII    EVQLVESGGGLVQPGGSLRLSCAASGFSFTGHWMNWVRQAPGKGLEWV
                                   =======
                                        +++++
                                       H1

          50  a                 70        80  abc      90        100      110
6G425     GYIDPSNGETTYNQKFKGKATLTVDTSSSTANVHLSSLTSDDSAVYFCAARGDYRYNGDWFFDVWGAGT
                            ## ### # ## ######  ### #   #                   #
F(ab)-1   GYIDPSNGETTYNQKFKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAARGDYRYNGDWFFDVWGQGT
           # #  ##  # ####                                    # # # ###  #
humIII    GMIHPSDSETRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAARGIYFY-GTTYFDYWGQGT
              ====                                            ===========
           +++++++++++++++++                                 +++++++++++++
                  H2                                               H3
```

FIG. 29

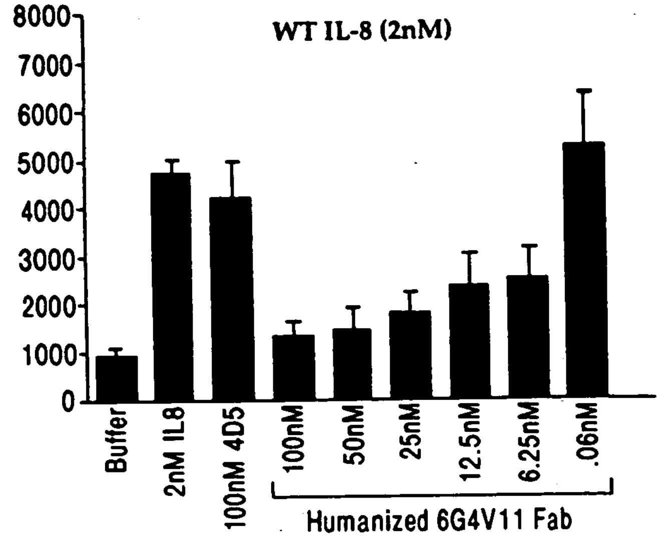


FIG. 30A

IC50~12nM

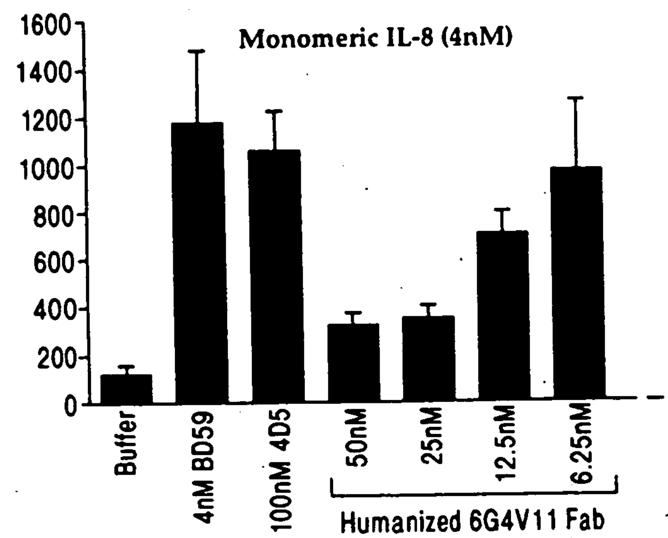


FIG. 30B

IC50~15nM

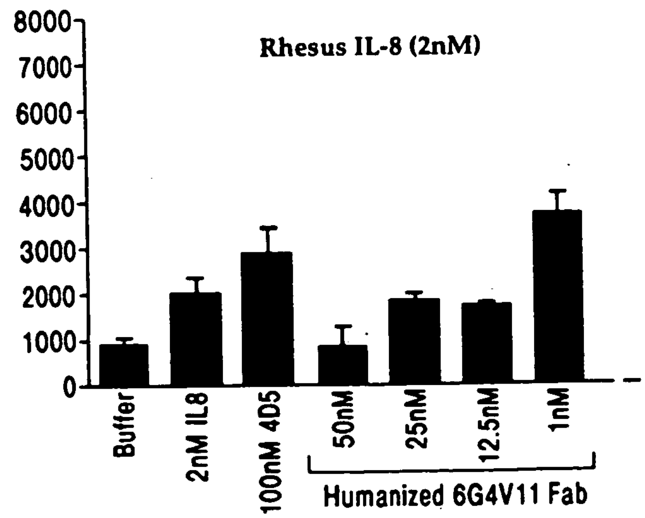


FIG. 30C

IC50~22nM

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLV**Q**SGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFT**L**SRDNSKNT**A**YLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

Amino Acid Sequence of the peptide linker and M13 Phage Coat (gene-III)

SGGGSGSGDFDYEKMANANKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVS
GLANGNGATGDFAGSSNSQMAQVGDGDNSPLMNNFRQYLPSLPQSVECRPFVFSAGKPY
EFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES

FIG. 31A

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N   I  A  F  L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A  Y  A  D   I  Q  M   T  Q  S   P  S  S  L   S  A  S   V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TAACACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
 18 R  V  T  I   T  C  R   S  S  Q   S  L  V  H   G  I  G   N  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L  H  W  Y   Q  Q  K   P  G  K   A  P  K  L   L  I  Y   K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N  R  F  S   G  V  P   S  R  F   S  G  S  G   S  G  T   D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L  T  I  S   S  L  Q   P  E  D   F  A  T  Y   Y  C  S   Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H  V  P  L   T  F  G   Q  G  T   K  V  E  I   K  R  T   V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P  S  V  F   I  F  P   P  S  D   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218 E  C  O
```

FIG. 31B

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

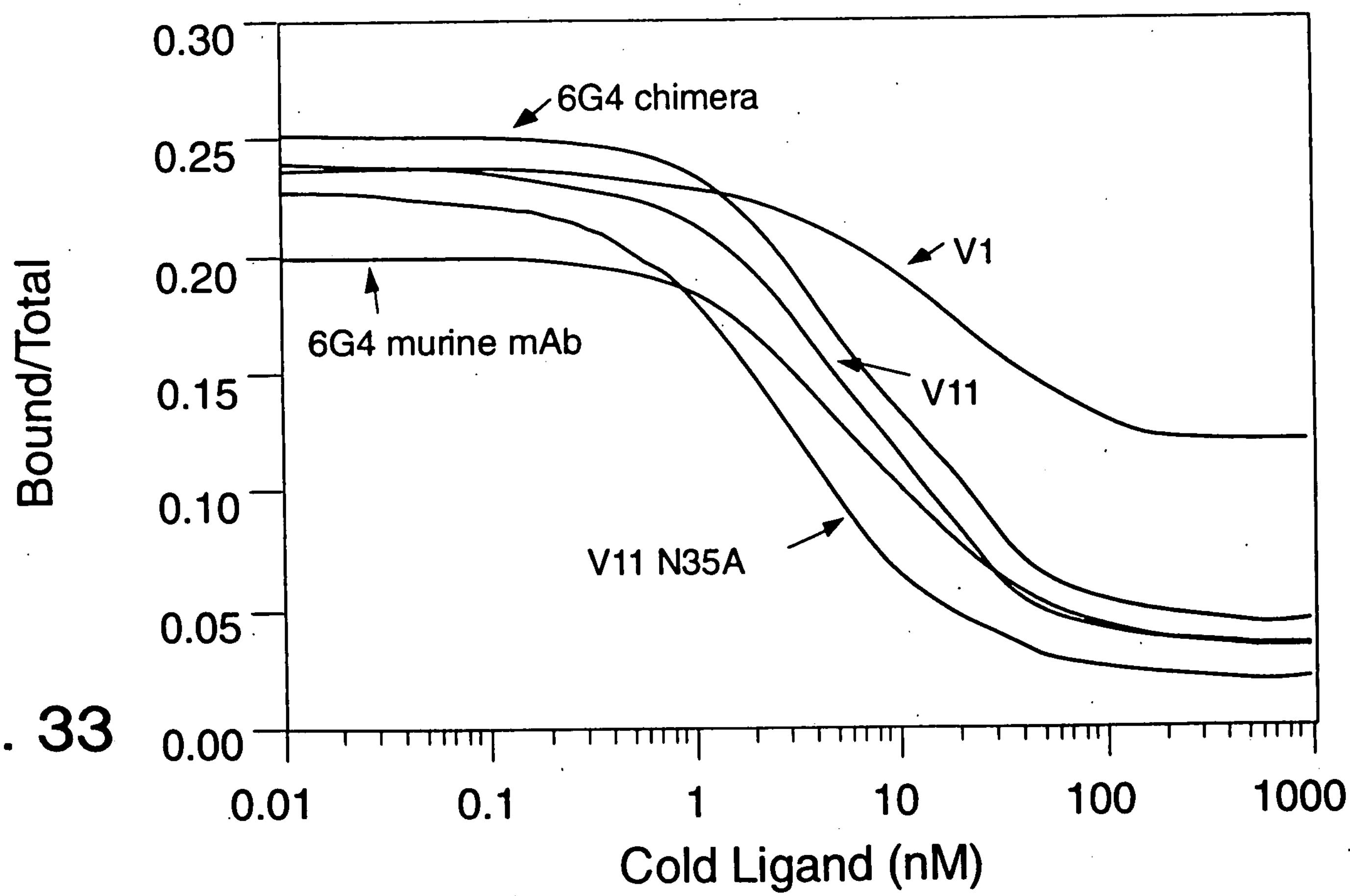
Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVKQ**A**PGKGLEWVGYIDPSNGETTYNQKFKGRFT**L**SRDNSKNT**A**YLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

FIG. 31C

FIG. 32

BEST AVAILABLE COPY



0.30
0.25
0.20
0.15
0.10
0.05
0.00
Bound/Total
6G4 chimera
V1
6G4 murine mAb
V11
V11 N35A
0.01
0.1
1
10
100
1000
Cold Ligand (nM)

FIG. 33

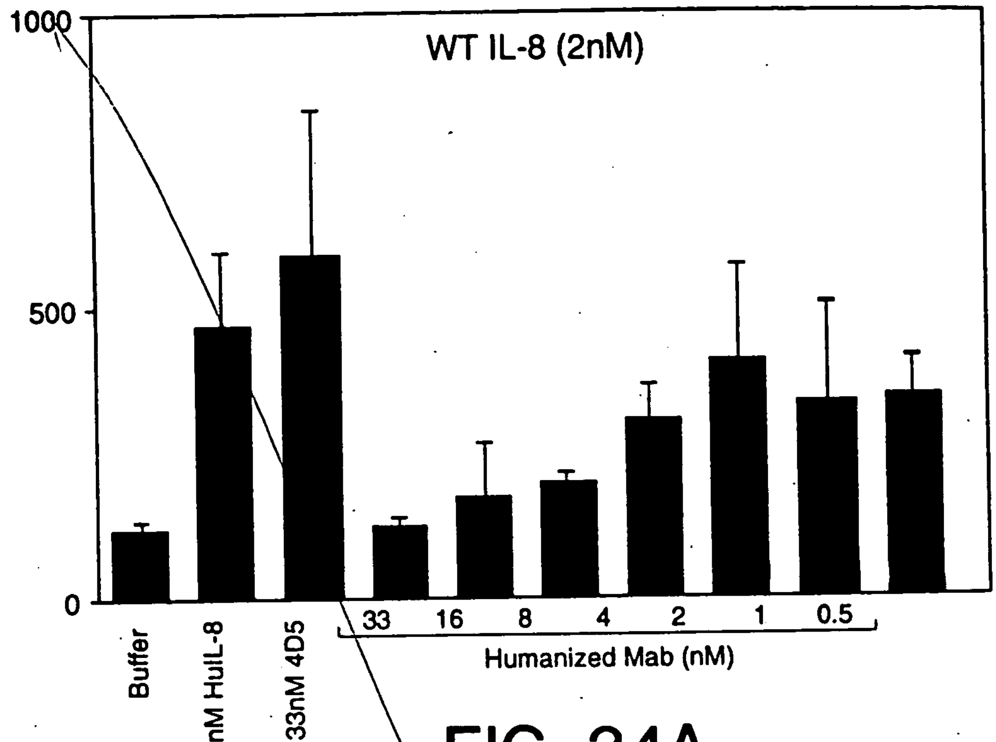


FIG. 34A

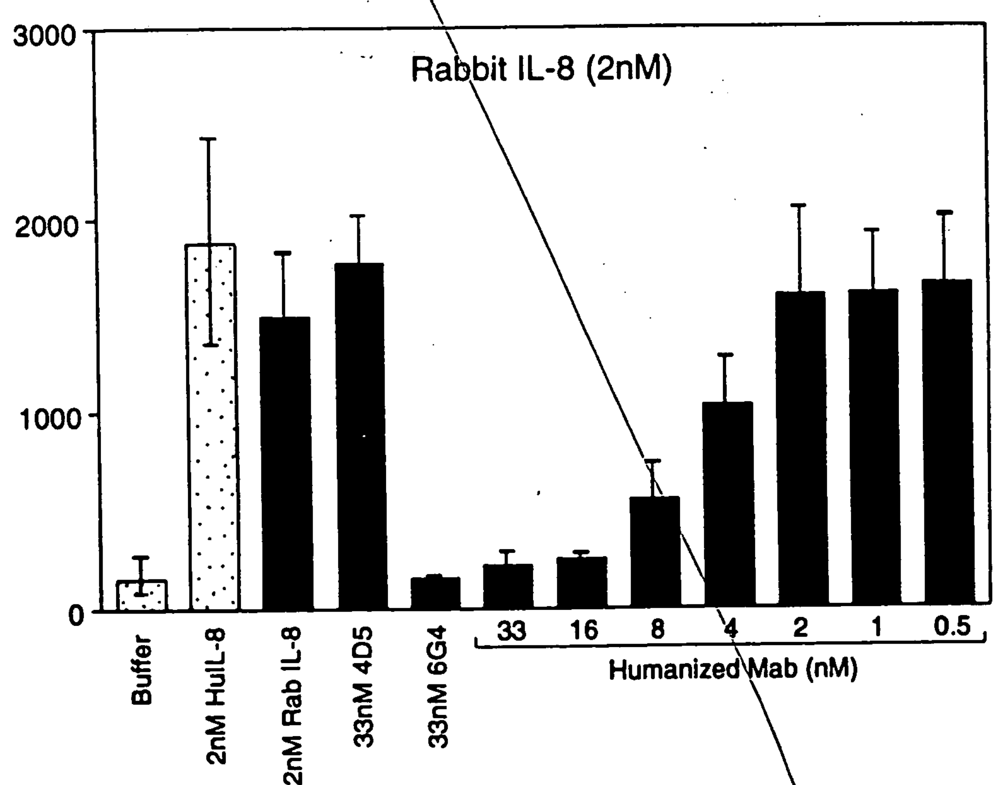


FIG. 34B

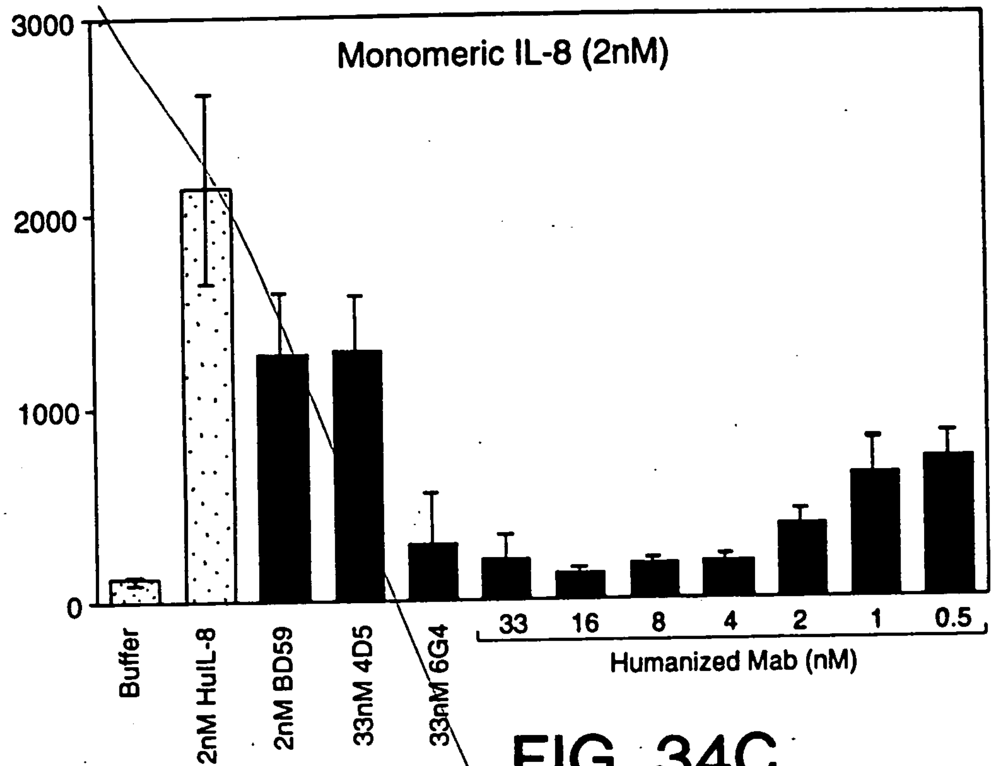


FIG. 34C

Ant
B4

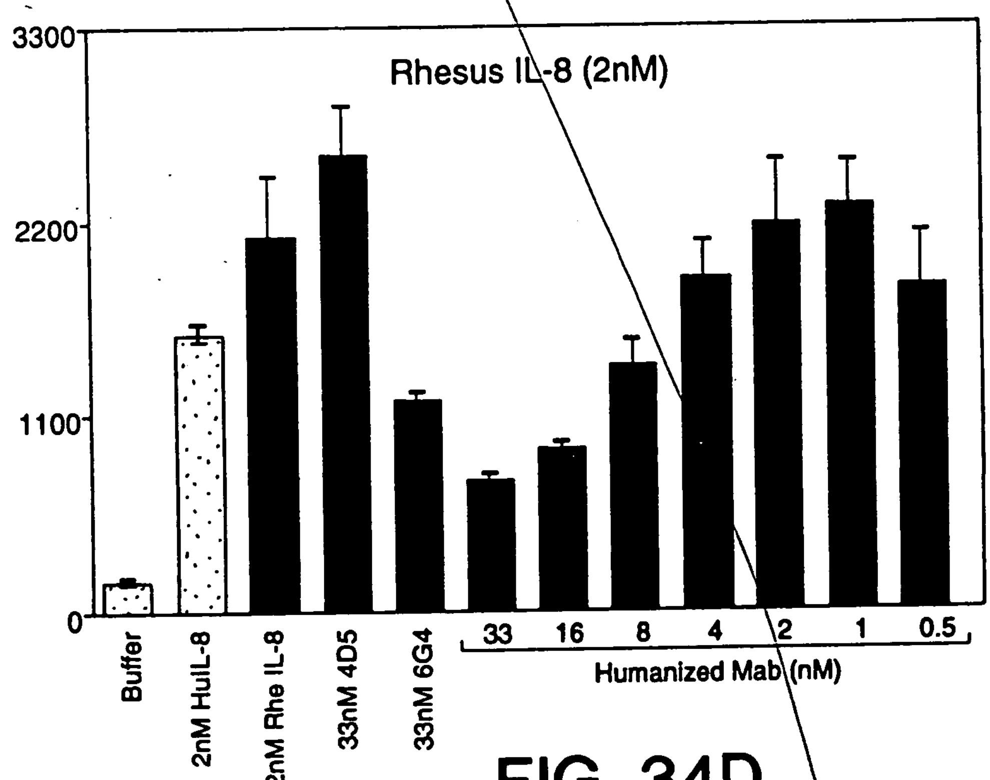


FIG. 34D

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIG**A**TY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

Amino Acid Sequence of the putative Pepsin Cleavage Site and GCN4 Leucine Zipper

CPPCPAPELLGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

FIG. 35

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A  Y  A  D   I  Q  M   T  Q  S   P  S  S  L   S  A  S   V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGCTACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
 18 R  V  T  I   T  C  R   S  S  Q   S  L  V  H   G  I  G   A  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L  H  W  Y   Q  Q  K   P  G  K   A  P  K  L   L  I  Y   K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N  R  F  S   G  V  P   S  R  F   S  G  S  G   S  G  T   D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L  T  I  S   S  L  Q   P  E  D   F  A  T  Y   Y  C  S   Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H  V  P  L   T  F  G   Q  G  T   K  V  E  I   K  R  T   V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P  S  V  F   I  F  P   P  S  D   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218 E  C  O
```

FIG. 36

```
781  AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
     TTTTCCCATA GATCTCCAAC TCCACTAAAA TACTTTTTCT TATAGCGTAA AGAAGAACGT
-1                                    M  K  K  N  I  A  F  L  L  A

841  TCTATGTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG AGGTTCAGCT AGTGCAGTCT
     AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
-11  S  M  F  V  F  S  I  A  T  N  A  Y  A  E  V  Q  L  V  Q  S

901  GGCGGTGGCC TGGTGCAGCC AGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
     CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
8    G  G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A  A  S  G  Y

961  TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
     AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTCCC GGACCTTACC
28   S  F  S  S  H  Y  M  H  W  V  R  Q  A  P  G  K  G  L  E  W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
     CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
48   V  G  Y  I  D  P  S  N  G  E  T  T  Y  N  Q  K  F  K  G  R

1081 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
     AAGTGAAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
68   F  T  L  S  R  D  N  S  K  N  T  A  Y  L  Q  M  N  S  L  R

1141 GCTGAGGACA CTGCCGTCTA TTACTGTGCA AGAGGGGATT ATCGCTACAA TGGTGACTGG
     CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
88   A  E  D  T  A  V  Y  Y  C  A  R  G  D  Y  R  Y  N  G  D  W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
     AAGAAGCTGC AGACCCCAGT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
108  F  F  D  V  W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
128  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

1321 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
148  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A

1381 CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
     GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
168  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
     TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC
188  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
     TTAGTGTTCG GGTCGTTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
208  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K

1561 ACTCACACAT GCCCGCCGTG CCCAGCACCA GAACTGCTGG GCGGCCGCAT GAAACAGCTA
     TGAGTGTGTA CGGGCGGCAC GGGTCGTGGT CTTGACGACC CGCCGGCGTA CTTTGTCGAT
228  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  R  M  K  Q  L
```

FIG. 37A

```
1621 GAGGACAAGG TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA
     CTCCTGTTCC AGCTTCTCGA TGAGAGGTTC TTGATGGTGG ATCTCTTACT TCACCGTTCT
 248 E  D  K  V   E  E  L   L  S  K   N  Y  H  L   E  N  E   V  A  R

1681 CTCAAAAAGC TTGTCGGGGA GCGCTAA
     GAGTTTTTCG AACAGCCCCT CGCGATT
 268 L  K  K  L   V  G  E   R  O
```

FIG. 37B

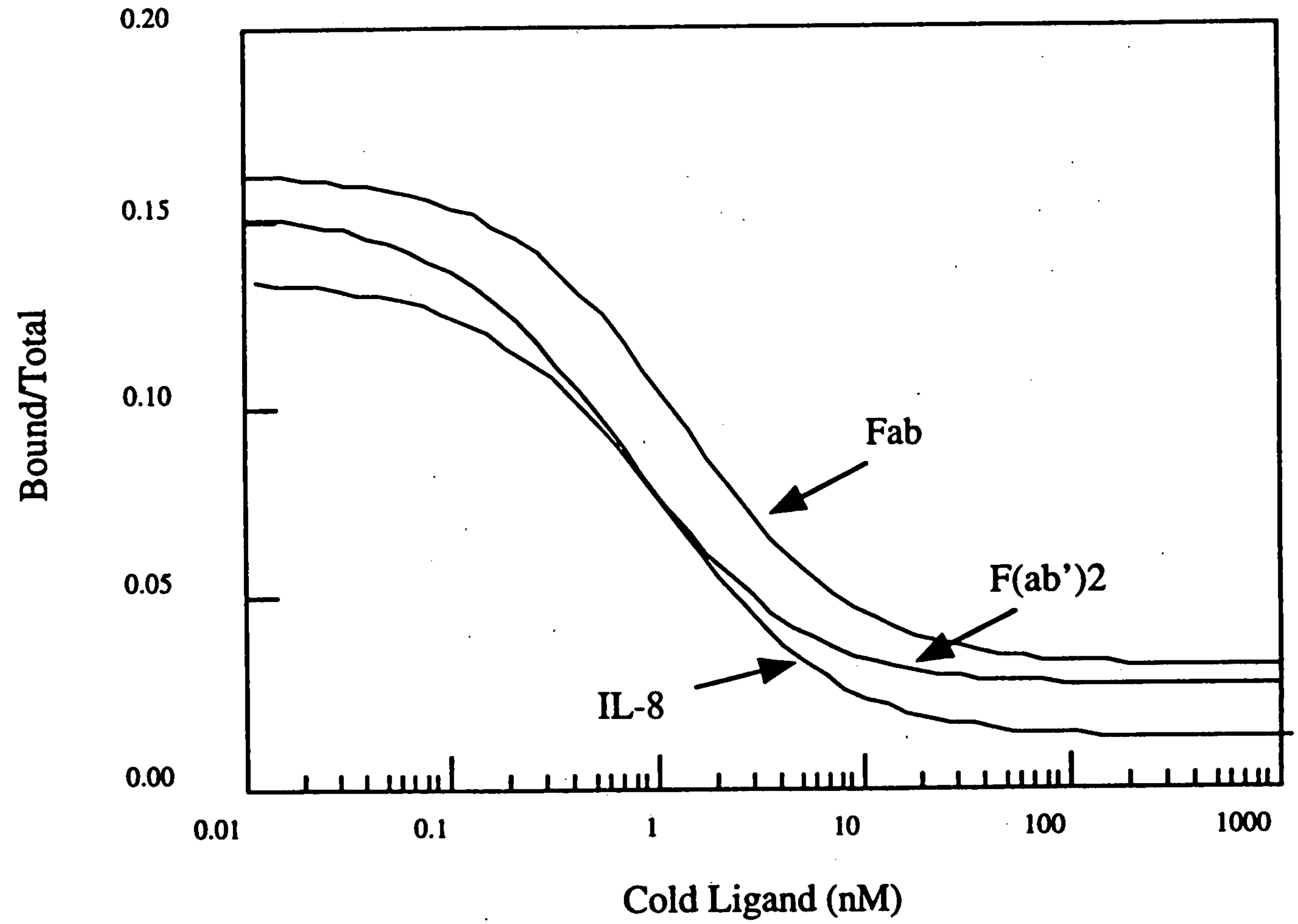

0.20
0.15
0.10
0.05
0.00
Bound/Total
0.01
0.1
1
10
100
1000
Cold Ligand (nM)
Fab
F(ab')2
IL-8

FIG. 38

00T2T0"1GEC8100

Ane
B5

FIG. 39

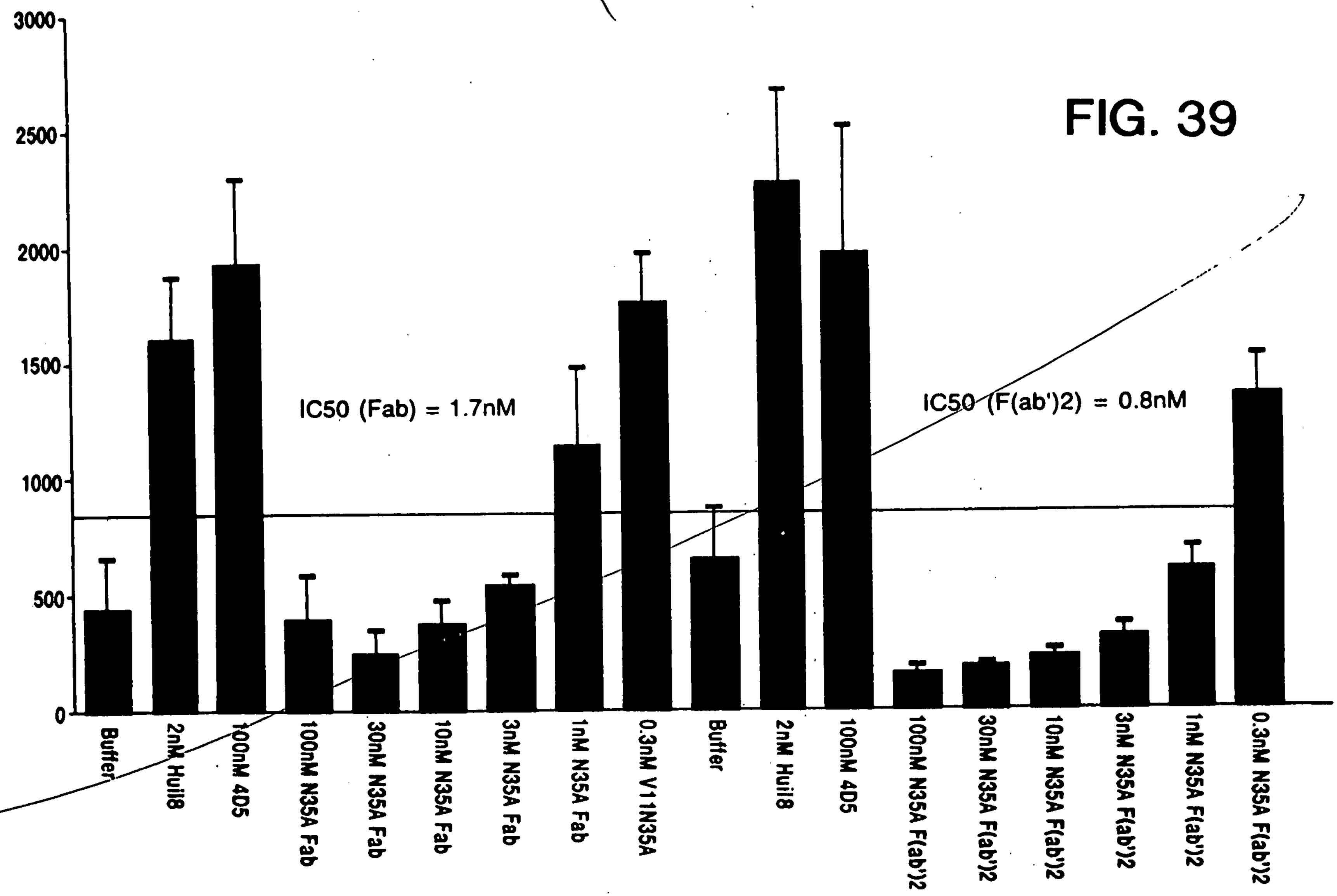
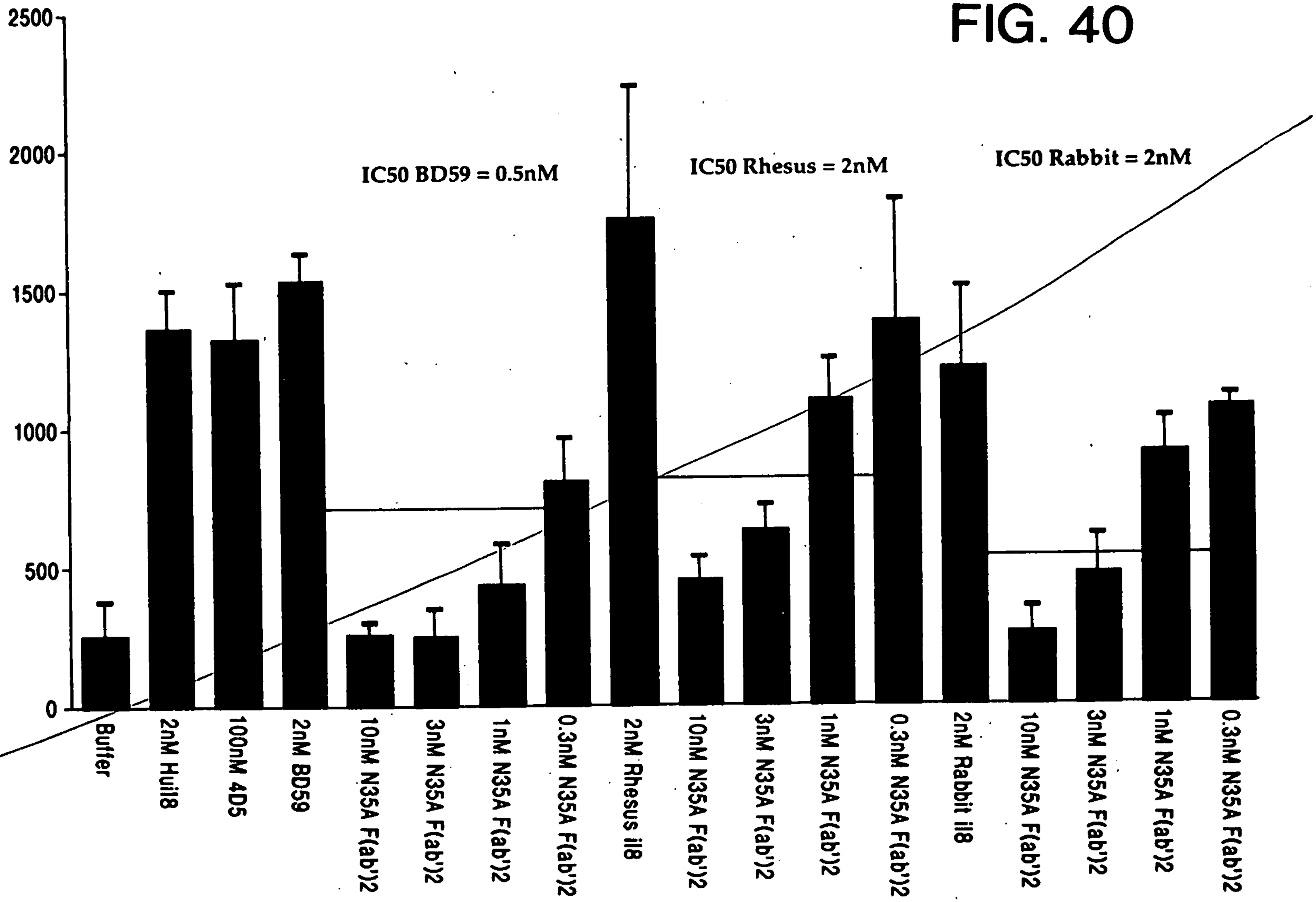


FIG. 40



IC50 BD59 = 0.5nM
IC50 Rhesus = 2nM
IC50 Rabbit = 2nM
2500
2000
1500
1000
500
0
Buffer
2nM Huil8
100nM 4D5
2nM BD59
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2
2nM Rhesus il8
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2
2nM Rabbit il8
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2

Ab
Bb

```
                                                                           aluI                    pleI
                                                                          hindIII              mboII  taqI
    ecoRI        pflMI                                     ddeI          tru9I                 earI/ksp632I
    apoI         bslI                      nlaIII      bsrDI             mseI cac8I        mboII   hinfI
  1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT
    CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA

               bspMI                                                                          sau3AI
            hinPI                                                                             mboI/ndeII[dam-]
            hhaI/cfoI                                                                         dpnI[dam+]
           mstI         aluI               mslI                    hinPI           aciI       dpnII[dam-]
           aviII/fspI hindIII            maeIII bsrDI              hhaI/cfoI      nspBII     bclI[dam-] mnlI
101 GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG
    CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC

                                                               thaI
                                                               fnuDII/mvnI
                                                           fnu4HI
                                                           bsoFI
                                                           bbvI      maeII
          rsaI                                          fnu4HI bstUI snaBI
      hinPI                                             bsoFI  bsh1236I                       mnlI
      hhaI/cfoI mnlI          cac8I                     bbvI  hinPI  bsaAI                 fokI
     haeII csp6I           sfaNI   bsmI                aluI   hhaI/cfoI                    sfaNI
201 GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA
    CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT

                                                                                                     aluI
                                                                                                    sstI
                                                                                                    sacI
                                                                                                    hgiJII
                                          haeIII/palI                                               hgiAI/aspHI
                                         mcrI                                                       ecl136II
                                         eagI/xmaIII/eclXI                                     ecoRI bsp1286
                                         eaeI                                              rmaI     bsiHKAI
                      aluI               cfrI                                              maeI     bmyI
         tru9I       pvuII               bsiEI   ahdI/eam1105I               tru9I         bfaI    taqI
         mseI        nspBII          maeIII    bsmAI                         mseI       maeIII apoI banII
301 AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GAATTCGAGC
    TTTTCAATTA GAAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT AAACATTGAT CTTAAGCTCG
```

FIG. 41A

```
          scrFI
          nciI
          mspI
          hpaII
          dsaV
         xmaI/pspAI
         smaI
         scrFI
         nciI
         dsaV
         cauII        xhoI
         bsaJI     mnlI
         avaI   sau3AI taqI
      rsaI      mboI/ndeII[dam-]
      csp6I     dpnI[dam+]
     nlaIV     nlaIV  paeR7I
     kpnI cauII dpnII[dam-]
     hgiCI     bstYI/xhoII
     banI bsaJI alwI[dam-]
     asp718    bamHI  avaI       hphI
     acc65I    alwI[dam-] mnlI  mnlI                          mboII   sfaNI
401 TCGGTACCCG GGGATCCTCT CGAGGTTGAG GTGATTTTAT GAAAAAGAAT ATCGCATTTC TTCTTGCATC TATGTTCGTT TTTTCTATTG CTACAAACGC
    AGCCATGGGC CCCTAGGAGA GCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCG
-23                                           M  K  K  N   I  A  F  L   L  A  S   M  F  V   F  S  I  A   T  N  A
      a mutation was found that inactivated the mluI site.  The penultimate nucleotide was changed fr G toT ^

                                 sstI
                                 sacI
                                 hgiJII
                                 hgiAI/aspHI
                                 ecl136II                                        bspMI
                                 bsp1286                                        scfI
                                 bsiHKAI                                        pstI
                          bsmFI  bmyI                           hphI          sse8387I           ddeI   nlaIII
                       bsrI  avaI aluI          mnlI            maeIII      bspMI             aluI   rsaI
          ecoRV     tth111I/aspI  banII       aciI             bstEII  hphI bsgI             hindIII csp6I
501 ATACGCTGAT ATCCAGATGA CCCAGTCCCC GAGCTCCCTG TCCGCCTCTG TGGGCGATAG GGTCACCATC ACCTGCAGGT CAAGTCAAAG CTTAGTACAT
    TATGCGACTA TAGGTCTACT GGGTCAGGGG CTCGAGGGAC AGGCGGAGAC ACCCGCTATC CCAGTGGTAG TGGACGTCCA GTTCAGTTTC GAATCATGTA
 -2  Y  A  D   I  Q  M  T   Q  S  P   S  S  L   S  A  S  V   G  D  R   V  T  I   T  C  R  S   S  Q  S   L  V  H
```

FIG. 41B

```
                                                      scrFI
                                                      mvaI                                                           tfiI
                                                      ecoRII                                                   hinfI      bsmFI
                                                      dsaV                                                   taqI    bpmI/gsuI[dcm-]
                                                      bstNI    aluI                                            claI/bsp106  pleI
                                     bsrI             apyI[dcm+]                                               bspDI[dam-]  hinfI
601 GGTATAGGTG CTACGTATTT ACACTGGTAT CAACAGAAAC CAGGAAAAGC TCCGAAACTA CTGATTTACA AAGTATCCAA TCGATTCTCT GGAGTCCCTT
    CCATATCCAC GATGCATAAA TGTGACCATA GTTGTCTTTG GTCCTTTTCG AGGCTTTGAT GACTAAATGT TTCATAGGTT AGCTAAGAGA CCTCAGGGAA
 32 G  I  G  A   T  Y  L   H  W  Y   Q  Q  K  P   G  K  A   P  K  L   L  I  Y  K   V  S  N   R  F  S   G  V  P  S

                 mspI
                 hpaII
                 bslI
                bsaWI
              sau3AI
              mboI/ndeII[dam-]
              dpnI[dam+]
              dpnII[dam-]
              alwI[dam-]                                        fnu4HI
             nlaIV                                              bsoFI
             bstYI/xhoII                                        bbvI
             bamHI                                             scfI      mboII                      rsaI
             alwI[dam-]  bsmFI                                 pstI      bpuAI                      csp6I
                                                               bsgI      bbsI                      scaI  nlaIII
701 CTCGCTTCTC TGGATCCGGT TCTGGGACGG ATTTCACTCT GACCATCAGC AGTCTGCAGC CAGAAGACTT CGCAACTTAT TACTGTTCAC AGAGTACTCA
    GAGCGAAGAG ACCTAGGCCA AGACCCTGCC TAAAGTGAGA CTGGTAGTCG TCAGACGTCG GTCTTCTGAA GCGTTGAATA ATGACAAGTG TCTCATGAGT
 66  R  F  S   G  S  G   S  G  T  D   F  T  L   T  I  S   S  L  Q  P   E  D  F   A  T  Y   Y  C  S  Q   S  T  H

                           styI
                            bsaJI
                         rsaI
                         csp6I
                        nlaIV
                        kpnI
                        hgiCI                sau3AI
      bsrBI             banI                 mboI/ndeII[dam-]  fnu4HI       mboII
      aciI              asp718               dpnI[dam+]        bsoFI       bpuAI          aciI
    bsmFI     maeII     acc65I               dpnII[dam-]       bbvI        bbsI     mboII
801 TGTCCCGCTC ACGTTTGGAC AGGGTACCAA GGTGGAGATC AAACGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC CATCTGATGA GCAGTTGAAA
    ACAGGGCGAG TGCAAACCTG TCCCATGGTT CCACCTCTAG TTTGCTTGAC ACCGACGTGG TAGACAGAAG TAGAAGGGCG GTAGACTACT CGTCAACTTT
 99 V  P  L   T  F  G  Q   G  T  K   V  E  I   K  R  T  V   A  A  P   S  V  F   I  F  P  P   S  D  E   Q  L  K
```

FIG. 41C

```
                                                                                                                     scrFI
                                                                                                                     mvaI
                                                                                                                     ecoRII
                                                                                                                     dsaV
                                                         haeIII/palI                                                 bstNI
       xmnI                     xmnI                   haeI      rsaI                        mnlI                   bsaJI
       asp700         cac8I     asp700                 mnlI      csp6I                       bslI         maeIII    apyI[dcm+]
901  TCTGGAACTG CTTCTGTTGT GTGCCTGCTG AATAACTTCT ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC
     AGACCTTGAC GAAGACAACA CACGGACGAC TTATTGAAGA TAGGGTCTCT CCGGTTTCAT GTCACCTTCC ACCTATTGCG GGAGGTTAGC CCATTGAGGG
132  S  G  T  A   S  V  V   C  L  L   N  N  F  Y   P  R  E   A  K  V   Q  W  K  V   D  N  A   L  Q  S   G  N  S  Q

                                                        fnu4HI
                                                        bsoFI         celII/espI
                                                    ddeI              blpI/bpu1102I
        maeIII                              scfI    mnlI bbvI     hgaI ddeI                                accI   cac8I
1001 AGGAGAGTGT CACAGAGCAG GACAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCTGA CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC
     TCCTCTCACA GTGTCTCGTC CTGTCGTTCC TGTCGTGGAT GTCGGAGTCG TCGTGGGACT GCGACTCGTT TCGTCTGATG CTCTTTGTGT TTCAGATGCG
166   E  S  V   T  E  Q   D  S  K  D   S  T  Y   S  L  S   S  T  L  T   L  S  K   A  D  Y   E  K  H  K   V  Y  A

                           cac8I
                          aluI
                         sstI
                         sacI
                         hgiJII
                         hgiAI/aspHI
                         ecl136II
                         bsp1286
                         bsiHKAI
                         bmyI                                                       mnlI                      rmaI
                     haeIII/palI                                                  sau3AI                      maeI
                     sau96I banII                                                 mboI/ndeII[dam-]            bfaI
                     asuI ddeI                                              aluI  dpnI[dam+]      hgaI      sau96I
        hphI        eco0109I/draII                                        tru9I   dpnII[dam-] mspI          haeIII/palI
       maeIII      alwNI[dcm-]           maeIII     aluI                  mseI    alwI[dam-]  hpaII sfaNI   asuI
1101 CTGCGAAGTC ACCCATCAGG GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAAGCT GATCCTCTAC GCCGGACGCA TCGTGGCCCT
     GACGCTTCAG TGGGTAGTCC CGGACTCGAG CGGGCAGTGT TTCTCGAAGT TGTCCCCTCT CACAATTCGA CTAGGAGATG CGGCCTGCGT AGCACCGGGA
199   C  E  V   T  H  Q  G   L  S  S   P  V  T   K  S  F  N  R  G  E   C  O
```

FIG. 41D

```
                   rmaI                          rmaI
                   maeI                          maeI
        rsaI       bfaI                          bfaI         hphI
        csp6I     speI                          xbaI mnlI    mnlI                                          mboII    sfaNI
1201 AGTACGCAAC TAGTCGTAAA AAGGGTATCT AGAGGTTGAG GTGATTTTAT GAAAAAGAAT ATCGCATTTC TTCTTGCATC TATGTTCGTT TTTTCTATTG
     TCATGCGTTG ATCAGCATTT TTCCCATAGA TCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC
-23                                                         M   K   K   N   I   A   F   L   L   A   S   M   F   V   F   S   I   A

                                                                      scrFI
                                                                      mvaI
                                                                      ecoRII
                                                                      dsaV
                                                            scrFI
          rsaI                                              mvaI    fnu4HI
         bsiWI/splI                                         ecoRII
        thaI                                                dsaV bstNI hgiJII                        aluI
        fnuDII/mvnI                                         bstNI  bsoFI    bsp1286                    alwNI[dcm-]
        bstUI                        rmaI                   apyI[dcm+] bsaJI bmyI                     fnu4HI
        bsh1236I                     maeI                   haeIII/palI  apyI[dcm+]                   bsoFI
       mluI csp6I   mnlI             bfaI                                                             bbvI
       aflIII      ddeI             aluI           aciI haeI        bbvI       banII
1301 CTACAAACGC GTACGCTGAG GTTCAGCTAG TGCAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC TGTGCAGCTT CTGGCTACTC
     GATGTTTGCG CATGCGACTC CAAGTCGATC ACGTCAGACC GCCACCGGAC CACGTCGGTC CCCCGAGTGA GGCAAACAGG ACACGTCGAA GACCGATGAG
-5    T   N   A   Y   A   E   V   Q   L   V   Q   S   G   G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A   A   S   G   Y   S

                                          scrFI
                                          nciI
                                          mspI
                                          hpaII
                                          dsaV
                                          cauII
                                          bslI
                                         xmaI/pspAI
                                         smaI
                                         scrFI
                                         nciI
                                         dsaV          scrFI
                                         cauII         mvaI
                                         bslI          ecoRII
```

FIG. 41E

```
                                     bsaJI          dsaV
                                     avaI           bstNI
          pleI                      bsaJI           bslI                           bslI
           hinfI          sau96I    sau96I          apyI[dcm+]                  sau3AI
         taqI             avaII     nlaIV          sau96I                 mboI/ndeII[dam-]
        xhoI              asuI      haeIII/palI    asuI                         dpnI[dam+]                 maeII
        paeR7I           nlaIV      asuI          eco0109I/draII          dpnII[dam-]            snaBI
        avaI maeIII    bsrI        eco0109I/draII haeIII/palI                  alwI[dam-]  hphI        bsaAI
1401 CTTCTCGAGT CACTATATGC ACTGGGTCCG TCAGGCCCCG GGTAAGGGCC TGGAATGGGT TGGATATATT GATCCTTCCA ATGGTGAAAC TACGTATAAT
     GAAGAGCTCA GTGATATACG TGACCCAGGC AGTCCGGGGC CCATTCCCGG ACCTTACCCA ACCTATATAA CTAGGAAGGT TACCACTTTG ATGCATATTA
  29  F  S  S   H  Y  M  H   W  V  R   Q  A  P   G  K  G  L   E  W  V   G  Y  I   D  P  S  N   G  E  T   T  Y  N

                                 thaI
                                 fnuDII/mvnI                scfI
             haeIII/palI         bstUI                      pstI
            sau96I               bsh1236I                   bsgI               cac8I   mnlI
            asuI                nruI                       bspMI            cac8I    ddeI  drdI
1501 CAAAAGTTCA AGGGCCGTTT CACTTTATCT CGCGACAACT CCAAAAACAC AGCATACCTG CAGATGAACA GCCTGCGTGC TGAGGACACT GCCGTCTATT
     GTTTTCAAGT TCCCGGCAAA GTGAAATAGA GCGCTGTTGA GGTTTTTGTG TCGTATGGAC GTCTACTTGT CGGACGCACG ACTCCTGTGA CGGCAGATAA
  62 Q  K  F  K   G  R  F   T  L  S   R  D  N  S   K  N  T   A  Y  L   Q  M  N  S   L  R  A   E  D  T   A  V  Y  Y

                                                                                                      sau96I
                                                                                                      haeIII/palI
                                                                                                     sau96I
                                                                                                     nlaIV
                                                                                                     hgiJII
                                                                   maeIII                            bsp1286
                                                                  bstEII                             bsp120I
                                                                scrFI                           bmyI
                                                                mvaI               mnlI              banII
                                          maeII                 ecoRII   bsaJI                  asuI
                                         hinlI/acyI             dsaV    bseRI                   apaI
                                         ahaII/bsaHI            bstNI     esp3I                   styI asuI
                              maeIII    taqI                   bsaJI hphI bsmBI             mnlI  bsaJI
           mnlI              hphI bsrI mboII aatII          nlaIV apyI[dcm+] bsmAI       haeIII/palI eco0109I/draII
1601 ACTGTGCAAG AGGGGATTAT CGCTACAATG GTGACTGGTT CTTCGACGTC TGGGGTCAAG GAACCCTGGT CACCGTCTCC TCGGCCTCCA CCAAGGGCCC
     TGACACGTTC TCCCCTAATA GCGATGTTAC CACTGACCAA GAAGCTGCAG ACCCCAGTTC CTTGGGACCA GTGGCAGAGG AGCCGGAGGT GGTTCCCGGG
  96  C  A  R   G  D  Y   R  Y  N  G   D  W  F   F  D  V   W  G  Q  G   T  L  V   T  V  S   S  A  S  T   K  G  P
                                                                     seq right is from p6G425chim2.fab2   ^
```

FIG. 41F

```
                                                                                  scrFI
                                                                                  mvaI
                                                                        scrFI
                                                                        mvaI      ecoRII
                          nlaIV                                         ecoRII
                          hgiCI                                         dsaV
                          banI                                          bstNI     ecoNI
                       scrFI                                  sau96I dsaV
                       mvaI                                         haeIII/palI   bstNI                     hphI
                       ecoRII                              fnu4HI    fnu4HI                          mspI
                       dsaV          hgiAI/aspHI           bsoFI bsaJI      bslI                     hpaII
               mboII   bstNI     bseRI     bsp1286                aciI  apyI[dcm+]                        cfr10I/bsrFI
              bpuAI    apyI[dcm+]  mnlI    bsiHKAI         bsp1286   asuI    bsoFI                        bsaWI  tth111I/aspI
              bbsI    bsaJI     mnlI       bmyI mnlI       bmyI nspBII bsaJI bbvI apyI[dcm+]        bslI  ageI maeIII
1701 ATCGGTCTTC CCCCTGGCAC CCTCCTCCAA GAGCACCTCT GGGGGCACAG CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG
     TAGCCAGAAG GGGGACCGTG GGAGGAGGTT CTCGTGGAGA CCCCCGTGTC GCCGGGACCC GACGGACCAG TTCCTGATGA AGGGGCTTGG CCACTGCCAC
 129  S  V  F   P  L  A  P   S  S  K   S  T  S   G  G  T  A   A  L  G   C  L  V   K  D  Y  F   P  E  P   V  T  V

                        hinPI
                        hhaI/cfoI
                       nlaIV                 hgiAI/aspHI
                       narI                  bsp1286
                       kasI                  bsiHKAI       mspI
                       hinlI/acyI        cac8I             hpaII
                       hgiCI          fnu4HI              scrFI                ddeI                  fnu4HI
                       haeII          bsoFI bmyI          nciI                 mnlI   pleI           bsoFI     maeIII     mnlI
                       banI           aciI  apaLI/snoI    dsaV                 eco81I hinfI     ddeI       hphI    bsp1286
                 ddeI ahaII/bsaHI nspBII alw44I/snoI cauII      scfI    bsu36I/mstII/sauI mnlI bbvI    bstEII  bmyI bpmI/gsuI[dcm-]
1801 TCGTGGAACT CAGGCGCCCT GACCAGCGGC GTGCACACCT TCCCGGCTGT CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGCCCT
     AGCACCTTGA GTCCGCGGGA CTGGTCGCCG CACGTGTGGA AGGGCCGACA GGATGTCAGG AGTCCTGAGA TGAGGGAGTC GTCGCACCAC TGGCACGGGA
 162 S  W  N  S   G  A  L   T  S  G   V  H  T  F   P  A  V   L  Q  S   S  G  L  Y   S  L  S   S  V  V   T  V  P  S

          aluI   nlaIV
        fnu4HI   hgiCI                                                 taqI          hgiJII
        bsoFI    banI                    tfiI                          salI          bsp1286
        bbvI    bsp1286                  hinfI                    styI hincII/hindII bmyI
     bstXI      bmyI                 maeII                        bsaJI accI         banII           maeIII
1901 CCAGCAGCTT GGGCACCCAG ACCTACATCT GCAACGTGAA TCACAAGCCC AGCAACACCA AGGTCGACAA GAAAGTTGAG CCCAAATCTT GTGACAAAAC
     GGTCGTCGAA CCCGTGGGTC TGGATGTAGA CGTTGCACTT AGTGTTCGGG TCGTTGTGGT TCCAGCTGTT CTTTCAACTC GGGTTTAGAA CACTGTTTTG
 196  S  S  L   G  T  Q   T  Y  I  C   N  V  N   H  K  P   S  N  T  K   V  D  K   K  V  E   P  K  S  C   D  K  T
```

FIG. 41G

```
                                               fnu4HI
                                               bsoFI
                                              haeIII/palI
                                            mcrI
                                            eagI/xmaIII/eclXI
                                            eaeI
                                            cfrI
                                            bsiEI                                     sapI
       cac8I                               notI               rmaI                    mboII
     nlaIII                                fnu4HI             maeI                    earI/ksp632I                rmaI
    nspI       bsp1286                     bsoFI   nlaIII     bfaI   tth111I/aspI                                 maeI
    nspHI  aciI bmyI                       aciI aciI       aluI mnlI       taqI   aluI                            bfaI
2001 TCACACATGC CCGCCGTGCC CAGCACCAGA ACTGCTGGGC GGCCGCATGA AACAGCTAGA GGACAAGGTC GAAGAGCTAC TCTCCAAGAA CTACCACCTA
     AGTGTGTACG GGCGGCACGG GTCGTGGTCT TGACGACCCG CCGGCGTACT TTGTCGATCT CCTGTTCCAG CTTCTCGATG AGAGGTTCTT GATGGTGGAT
229  H  T  C   P  P  C  P   A  P  E   L  L  G   G  R  M  K   Q  L  E   D  K  V   E  E  L  L   S  K  N   Y  H  L
       ^junction between antibody and leucine zipper

                                                                                           scrFI
                                                 sphI                                      nciI
                                              ddeI nlaIII                                  mspI
                                             celII/espI        rmaI                        hpaII
                                             blpI/bpu1102I     maeI                        dsaV
                                           hinPI   nspI        bfaI  bsmFI                 cauII
                                           hhaI/cfoI         sau96I  pleI               aciI
               pleI      aluI             haeII    nspHI     haeIII/palI                fnu4HI
               hinfI   hindIII            eco47III cac8I     asuI    hinfI              bsoFI
2101 GAGAATGAAG TGGCAAGACT CAAAAAGCTT GTCGGGGAGC GCTAAGCATG CGACGGCCCT AGAGTCCCTA ACGCTCGGTT GCCGCCGGGC GTTTTTTATT
     CTCTTACTTC ACCGTTCTGA GTTTTTCGAA CAGCCCCTCG CGATTCGTAC GCTGCCGGGA TCTCAGGGAT TGCGAGCCAA CGGCGGCCCG CAAAAAATAA
262  E  N  E  V   A  R  L   K  K  L   V  G  E  R   O

      tru9I                               aluI
      mseI                           taqI  hindIII                                             nlaIV
     hpaI   nlaIII                  claI/bsp106 tru9I                   tru9I                  hgiCI
     hincII/hindII     aluI         bspDI[dam-] mseI   aciI             mseI                   banI
2201 GTTAACTCAT GTTTGACAGC TTATCATCGA TAAGCTTTAA TGCGGTAGTT TATCACAGTT AAATTGCTAA CGCAGTCAGG CACCGTGTAT GAAATCTAAC
     CAATTGAGTA CAAACTGTCG AATAGTAGCT ATTCGAAATT ACGCCATCAA ATAGTGTCAA TTTAACGATT GCGTCAGTCC GTGGCACATA CTTTAGATTG
```

FIG. 41H

```
                                        sfaNI                                                    haeIII/palI
                                     scrFI                                              sau96I
                                     mvaI                                                  scrFI
                                     ecoRII                                          nciI
                                     dsaV                                     rsaI   mspI mnlI
                          nlaIV      bstNI                                          csp6I  hpaII
                     mnlI hgiCI     bsaJI                                        mspI      dsaV bslI
     hinPI           bsaJI      hphI apyI[dcm+]                                  hpaII     cauII       ecoRV
     hhaI/cfoI   fokI     banI maeIII    fokI   scfI                            cfr10I/bsrFI asuI   aciI
2301 AATGCGCTCA TCGTCATCCT CGGCACCGTC ACCCTGGATG CTGTAGGCAT AGGCTTGGTT ATGCCGGTAC TGCCGGGCCT CTTGCGGGAT ATCGTCCATT
     TTACGCGAGT AGCAGTAGGA GCCGTGGCAG TGGGACCTAC GACATCCGTA TCCGAACCAA TACGGCCATG ACGGCCCGGA GAACGCCCTA TAGCAGGTAA

                                       hinPI
                                       hhaI/cfoI
                                     rmaI
                                     maeI
                                    nheI                                                                     fnu4HI
                                 fnu4HI haeII                                 hinPI            hgiAI/aspHI   bsoFI
                                 bsoFI  eco47III                              hhaI/cfoI        bsp1286    aciI  haeIII/palI
                     maeIII      bbvI bfaI                                   mstI  bslI        bsiHKAI  mcrI   eaeI
        sfaNI   bsrI         cac8I    cac8I             sfaNI                aviII/fspI        bmyI     bsiEI  cfrI
2401 CCGACAGCAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT ATATGCGTTG ATGCAATTTC TATGCGCACC CGTTCTCGGA GCACTGTCCG ACCGCTTTGG
     GGCTGTCGTA GCGGTCAGTG ATACCGCACG ACGATCGCGA TATACGCAAC TACGTTAAAG ATACGCGTGG GCAAGAGCCT CGTGACAGGC TGGCGAAACC

                                                                                      mnlI
                                                                                   sau3AI
                                                       sau3AI                      mboI/ndeII[dam-]
                                                       mboI/ndeII[dam-]            dpnI[dam+]
                                                       dpnI[dam+]                  dpnII[dam-]
                                                       dpnII[dam-]                 alwI[dam-]
     aciI                                           thaI                          nlaIV
     fnu4HI                                         fnuDII/mvnI                   bstYI/xhoII     hgaI
     bsoFI                                          bstUI  nlaIII                 bamHI        mspI
     aciI   bsrI      cac8I          nlaIV     taqI bsh1236I          bslI        alwI[dam-]   hpaII  sfaNI
2501 CCGCCGCCCA GTCCTGCTCG CTTCGCTACT TGGAGCCACT ATCGACTACG CGATCATGGC GACCACACCC GTCCTGTGGA TCCTCTACGC CGGACGCATC
     GGCGGCGGGT CAGGACGAGC GAAGCGATGA ACCTCGGTGA TAGCTGATGC GCTAGTACCG CTGGTGTGGG CAGGACACCT AGGAGATGCG GCCTGCGTAG
```

FIG. 41I

```
                     hinPI
                      hhaI/cfoI
                     nlaIV
                     narI
                     kasI                  hinPI
                     hinlI/acyI             hhaI/cfoI
        mspI         hgiCI                 nlaIV
        hpaII        haeII                 narI                                         hgiJII
       naeI        mspI                    kasI                                         bsp1286
       cfr10I/bsrFI banI                   hinlI/acyI                                   bmyI
       cac8I    sgrAI                      hgiCI                                        banII
      haeIII/palI hpaII                    haeII                                    sau3AI cac8I          rcaI  hinPI
     eaeI      hphI ahaII/bsaHI            banI                                     mboI/ndeII[dam-]   hgiJII     haeII
     cfrI  sfaNI cfr10I/bsrFI              ahaII/bsaHI                              dpnI[dam+]         bsp1286    eco47III
     cfrI  sfaNI cfr10I/bsrFI    aciI   cac8I                    hphI          mboII[dam-]             bmyI bspHI hhaI/cfoI
                                                                                                       banII nlaIII
2601 GTGGCCGGCA TCACCGGCGC CACAGGTGCG GTTGCTGGCG CCTATATCGC CGACATCACC GATGGGGAAG ATCGGGCTCG CCACTTCGGG CTCATGAGCG
     CACCGGCCGT AGTGGCCGCG GTGTCCACGC CAACGACCGC GGATATAGCG GCTGTAGTGG CTACCCCTTC TAGCCCGAGC GGTGAAGCCC GAGTACTCGC

                                           scrFI
                                           nciI
                                           mspI            hinPI
                                           hpaII           hhaI/cfoI
                                     dsaI  dsaV           nlaIV
                                     bslI  cauII          narI
                                sau96I  haeIII/palI      kasI                                      fnu4HI
                                nlaIV   eaeI             hinlI/acyI                                bsoFI hgiAI/aspHI
                                haeIII/palI              hgiCI                                     aciI  bsp1286
                                asuI bsaJI bsaJI         haeII                                    fnu4HI  bsiHKAI
                               ecoO109I/draII            banI                                     bsoFI   bmyI
                             cac8I bslI cfrI    bsmFI     ahaII/bsaHI    cac8I         bslI aciI  aciI          haeIII/palI
2701 CTTGTTTCGG CGTGGGTATG GTGGCAGGCC CCGTGGCCGG GGGACTGTTG GGCGCCATCT CCTTGCACGC ACCATTCCTT GCGGCGGCGG TGCTCAACGG
     GAACAAAGCC GCACCCATAC CACCGTCCGG GGCACCGGCC CCCTGACAAC CCGCGGTAGA GGAACGTGCG TGGTAAGGAA CGCCGCCGCC ACGAGTTGCC

                                                                                                              mspI
                 fnu4HI                                                                                       hpaII
                 bsoFI   ecoNI      pleI                                                                     bsaWI
     mnlI  bslI   bsrI bbvI   bslI       hinfI          hgaI      sfaNI                      bsrI  aluI bslI
2801 CCTCAACCTA CTACTGGGCT GCTTCCTAAT GCAGGAGTCG CATAAGGGAG AGCGTCGTCC GATGCCCTTG AGAGCCTTCA ACCCAGTCAG CTCCTTCCGG
     GGAGTTGGAT GATGACCCGA CGAAGGATTA CGTCCTCAGC GTATTCCCTC TCGCAGCAGG CTACGGGAAC TCTCGGAAGT TGGGTCAGTC GAGGAAGGCC
```

FIG. 41J

```
                                                                                                          fnu4HI
      aciI                                                                                             mspI  hinPI
     thaI                                                                                             naeI  haeII
     fnuDII/mvnI                                                                                      cfr10I/bsrFI
     bstUI   nlaIII                                                                                   cac8I eco47III
     bsh1236I              aciI               mboII                                               nlaIV    bsoFI
    hinPI   bcgI          fnu4HI             bpuAI                                                hgiCI    bbvI
    hhaI/cfoI             bsoFI              bbsI             nlaIII                              banI hpaII hhaI/cfoI           mnlI
2901 TGGGCGCGGG GCATGACTAT CGTCGCCGCA CTTATGACTG TCTTCTTTAT CATGCAACTC GTAGGACAGG TGCCGGCAGC GCTCTGGGTC ATTTTCGGCG
     ACCCGCGCCC CGTACTGATA GCAGCGGCGT GAATACTGAC AGAAGAAATA GTACGTTGAG CATCCTGTCC ACGGCCGTCG CGAGACCCAG TAAAAGCCGC

                      thaI
                      fnuDII/mvnI                                                                                  sau96I
                      bstUI          haeIII/palI                                                                   nlaIV
      aciI            bsh1236I  sau3AI                                                                             avaII
   sau96I            hinPI      mboI/ndeII[dam-]                                                                   asuI
   avaII             hhaI/cfoI  dpnI[dam+]          aciI         tfiI                                           bsrI     aciI
   asuI           bpmI/gsuI[dcm-] dpnII[dam-]     cac8I          hinfI     cac8I mnlI                        maeIII  bsmFI
3001 AGGACCGCTT TCGCTGGAGC GCGACGATGA TCGGCCTGTC GCTTGCGGTA TTCGGAATCT TGCACGCCCT CGCTCAAGCC TTCGTCACTG GTCCCGCCAC
     TCCTGGCGAA AGCGACCTCG CGCTGCTACT AGCCGGACAG CGAACGCCAT AAGCCTTAGA ACGTGCGGGA GCGAGTTCGG AAGCAGTGAC CAGGGCGGTG

                                          mcrI
                                          eagI/xmaIII/eclXI
                                          eaeI    hinPI
                                          cfrI    hhaI/cfoI                                        thaI
                                mspI      bsiEI  thaI                                          hgaI
                               naeI      fnu4HI  fnuDII/mvnI                                 thaI  fnuDII/mvnI
                               cfr10I/bsrFI      bstUI                                       fnuDII/mvnI
                  haeIII/palI hpaII      bsoFI   bsh1236I                                    bstUI bstUI
    maeII        haeI          cac8I     aciI  hgaI                                          bsh1236I mnlI     haeI
   psp1406I     cac8I          bglI nlaIII haeIII/palI     maeII    cac8I    nruI   bsh1236I fokI haeIII/palI
3101 CAAACGTTTC GGCGAGAAGC AGGCCATTAT CGCCGGCATG GCGGCCGACG CGCTGGGCTA CGTCTTGCTG GCGTTCGCGA CGCGAGGCTG GATGGCCTTC
     GTTTGCAAAG CCGCTCTTCG TCCGGTAATA GCGGCCGTAC CGCCGGCTGC GCGACCCGAT GCAGAACGAC CGCAAGCGCT GCGCTCCGAC CTACCGGAAG
```

FIG. 41K

```
                                                                            bspMI
                                              thaI                        scrFI
                             fnu4HI           fnuDII/mvnI                 mvaI
                             bsoFI            bstUI                       ecoRII
         mboII               aciI         cac8I       haeI                dsaV
     tfiI                 mspI   mslI   sfaNI  bsh1236I  haeIII/palI      bstNI
     hinfI                hpaII sfaNI  fokI   aciI   cac8I   nlaIII       apyI[dcm+]                  bsmFI aluI    alwI[dam-]
3201 CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG
     GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCGCAACGT CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC

        fnu4HI
        bsoFI
        aciI
       thaI                                    sau96I
       fnuDII/mvnI                              avaII
       bstUI                                 bsrI         sau3AI                    mnlI
     cac8I                               sau3AI asuI      mboI/ndeII[dam-]          bsaJI   hgiAI/aspHI
sau3AI bsh1236I                          mboI/ndeII[dam-]                         aciI      bsp1286
mboI/ndeII[dam-]                         dpnI[dam+] nspBII    maeIII             fnu4HI     bsiHKAI
dpnI[dam+]                               dpnII[dam-]      dpnI[dam+]             bsoFI      bmyI
dpnII[dam-]                            taqI[dam-]    aciI dpnII[dam-]            bglI     cac8I   nlaIII          nlaIII
3301 GATCGCTCGC GGCTCTTACC AGCCTAACTT CGATCACTGG ACCGCTGATC GTCACGGCGA TTTATGCCGC CTCGGCGAGC ACATGGAACG GGTTGGCATG
     CTAGCGAGCG CCGAGAATGG TCGGATTGAA GCTAGTGACC TGGCGACTAG CAGTGCCGCT AAATACGGCG GAGCCGCTCG TGTACCTTGC CCAACCGTAC

          fnu4HI
          bsoFI
       hinPI
       hhaI/cfoI
      nlaIV                                                              haeIII/palI
      narI                                                              sau96I                         fnu4HI
      kasI                                                             scrFI                           bsoFI
      hinlI/acyI                                        thaI           nciI                            aciI
      hgiCI                              thaI           fnuDII/mvnI    mspI                          mspI      mnlI
      haeII                              fnuDII/mvnI                   hpaII                         hpaII nlaIV
      banI aciI                          bstUI          bstUI          dsaV                         naeI   hgiCI
      ahaII/bsaHI                        bsh1236I       bsh1236I  nlaIV asuI    taqI                cfr10I/bsrFI
                                    mnlI aciI      hgaI aciI   nlaIII  cauII   mnlI                 cac8I  banI
3401 GATTGTAGGC GCCGCCCTAT ACCTTGTCTG CCTCCCCGCG TTGCGTCGCG GTGCATGGAG CCGGGCCACC TCGACCTGAA TGGAAGCCGG CGGCACCTCG
     CTAACATCCG CGGCGGGATA TGGAACAGAC GGAGGGGCGC AACGCAGCGC CACGTACCTC GGCCCGGTGG AGCTGGACTT ACCTTCGGCC GCCGTGGAGC
```

FIG. 41L

```
                                                                     hinPI                                hgaI
                                                                     hhaI/cfoI                           thaI aciI
              hphI                                                  mstI     pflMI                       fnuDII/mvnI
           tfiI        pflMI                                        aviII/fspI     styI                  bstUI
           hinfI       bslI      nlaIV             aciI         bsmI        bslI  bsaJI                  bsh1236I
3501 CTAACGGATT CACCACTCCA AGAATTGGAG CCAATCAATT CTTGCGGAGA ACTGTGAATG CGCAAACCAA CCCTTGGCAG AACATATCCA TCGCGTCCGC
     GATTGCCTAA GTGGTGAGGT TCTTAACCTC GGTTAGTTAA GAACGCCTCT TGACACTTAC GCGTTTGGTT GGGAACCGTC TTGTATAGGT AGCGCAGGCG

                                                        haeIII/palI
                                                       mscI/balI
                                                       haeI
                                                     scrFI
                                                     mvaI dsaI                                              mspI
                                                     ecoRII                                           hpaII
                                                     dsaV                                            scrFI
                                                     bstNI                                                nciI
                        fnu4HI                       bslI bsaJI           sau3AI                          dsaV
                       thaI hinPI                    apyI[dcm+]           mboI/ndeII[dam-]             sau96I
                 fnu4HI bsoFI                     sau96I            dpnI[dam+]            nlaIV
                 bsoFI fnuDII/mvnI                avaII      hinPI  dpnII[dam-]           avaII            cac8I
             fnu4HI    bstUI                      asuI eaeI  hhaI/cfoI  hgiAI/aspHI       asuI     rmaI
             bsoFI  cac8I   hhaI/cfoI   fnu4HI   ppuMI      mstI nlaIII bsp1286          ppuMI     maeI
             bbvI aciI bsh1236I   avaI  bsoFI    nlaIV cfrI       aviII/fspI  bsiHKAI            ecoO109I/draII
        bpmI/gsuI[dcm-] aciI  sfaNI     bbvI     ecoO109I/draII  mslI   bmyI            mnlI cauII-bfaI   aciI
3601 CATCTCCAGC AGCCGCACGC GGCGCATCTC GGGCAGCGTT GGGTCCTGGC CACGGGTGCG CATGATCGTG CTCCTGTCGT TGAGGACCCG GCTAGGCTGG
     GTAGAGGTCG TCGGCGTGCG CCGCGTAGAG CCCGTCGCAA CCCAGGACCG GTGCCCACGC GTACTAGCAC GAGGACAGCA ACTCCTGGGC CGATCCGACC

                                                                           fnu4HI
                                                 cac8I                     bsoFI
                                               thaI                        bbvI
                                      hphI     fnuDII/mvnI              fnu4HI
                                  tfiI         bstUI                    bsoFI
                  bsrI            hinfI        bsh1236I  maeII          bbvI        maeII       ddeI        nlaIII
3701 CGGGGTTGCC TTACTGGTTA GCAGAATGAA TCACCGATAC GCGAGCGAAC GTGAAGCGAC TGCTGCTGCA AAACGTCTGC GACCTGAGCA ACAACATGAA
     GCCCCAACGG AATGACCAAT CGTCTTACTT AGTGGCTATG CGCTCGCTTG CACTTCGCTG ACGACGACGT TTTGCAGACG CTGGACTCGT TGTTGTACTT
```

FIG. 41M

```
                                                                  sau3AI
                                                                  mboI/ndeII[dam-]
                                                                  mamI[dam-]
                                                                  dpnI[dam+]
                                                                  dpnII[dam-]
                                                                 bstYI/xhoII
                                                                 alwI[dam-]
                                                               mspI
                                                               hpaII
                                     aciI                     mroI bsaBI[dam-]   fnu4HI
                                    thaI                      bspMII             bsoFI
       mboII                        fnuDII/mvnI hinPI         bspEI[dam-]        bbvI
      bpuAI                         bstUI       hhaI/cfoI     bsaWI   sfaNI   sfaNI
      bbsI                          bsh1236I  haeII  mslI     accIII[dam-]    fokI  cac8I
3801 TGGTCTTCGG TTTCCGTGTT TCGTAAAGTC TGGAAACGCG GAAGTCAGCG CCCTGCACCA TTATGTTCCG GATCTGCATC GCAGGATGCT GCTGGCTACC
     ACCAGAAGCC AAAGGCACAA AGCATTTCAG ACCTTTGCGC CTTCAGTCGC GGGACGTGGT AATACAAGGC CTAGACGTAG CGTCCTACGA CGACCGATGG

                                                              aciI
                                 cac8I                     bsmFI   fokI
                               hinPI                      sau96I  sfaNI
                               hhaI/cfoI                  nlaIV aciI
                        tru9I haeII                       avaII fnu4HI      bsrI
                        mseI  eco47III     ddeI           asuI  bsoFI   aciI         mnlI
3901 CTGTGGAACA CCTACATCTG TATTAACGAA GCGCTGGCAT TGACCCTGAG TGATTTTTCT CTGGTCCCGC CGCATCCATA CCGCCAGTTG TTTACCCTCA
     GACACCTTGT GGATGTAGAC ATAATTGCTT CGCGACCGTA ACTGGGACTC ACTAAAAAGA GACCAGGGCG GCGTAGGTAT GGCGGTCAAC AAATGGGAGT

                      nspI
                 scrFI
                 nciI
                 mspI
          bsrI   hpaII
          bslI   dsaV nlaIII                      mnlI
       maeII     cauII                          fokI
      psp1406I maeIII nspHI      maeIII       sfaNI                        nlaIII apoI  bslI
4001 CAACGTTCCA GTAACCGGGC ATGTTCATCA TCAGTAACCC GTATCGTGAG CATCCTCTCT CGTTTCATCG GTATCATTAC CCCCATGAAC AGAAATTCCC
     GTTGCAAGGT CATTGGCCCG TACAAGTAGT AGTCATTGGG CATAGCACTC GTAGGAGAGA GCAAAGTAGC CATAGTAATG GGGGTACTTG TCTTTAAGGG
```

FIG. 41N

```
                                                              cac8I
                                                             sau96I
                                                     tru9I   haeIII/palI
                  sfaNI                              mseI    asuI                      tru9I
              mnlI       maeIII               aciI bslI  nlaIII aciI                   mseI      bpmI/gsuI[dcm-]
4101 CCTTACACGG AGGCATCAAG TGACCAAACA GGAAAAAACC GCCCTTAACA TGGCCCGCTT TATCAGAAGC CAGACATTAA CGCTTCTGGA GAAACTCAAC
     GGAATGTGCC TCCGTAGTTC ACTGGTTTGT CCTTTTTTGG CGGGAATTGT ACCGGGCGAA ATAGTCTTCG GTCTGTAATT GCGAAGACCT CTTTGAGTTG

                                                                                  fnu4HI   thaI
                                                                                  bsoFI    fnuDII/mvnI
                                                                                aluI       bstUI
                                                                               pvuII      hinPI
              aciI                                                             nspBII     hhaI/cfoI
             thaI                                                             fnu4HI     thaI
             fnuDII/mvnI                 xmnI                                 bsoFI      fnuDII/mvnI
             bstUI                       tfiI                                 bcgI       bstUI
             bsh1236I                    hinfI                                bbvI    mnlI bsh1236I
      aluI hgaI  fokI                    asp700          mslI        aluI    aciI  bbvI   bsh1236I   hphI      hphI
4201 GAGCTGGACG CGGATGAACA GGCAGACATC TGTGAATCGC TTCACGACCA CGCTGATGAG CTTTACCGCA GCTGCCTCGC GCGTTTCGGT GATGACGGTG
     CTCGACCTGC GCCTACTTGT CCGTCTGTAG ACACTTAGCG AAGTGCTGGT GCGACTACTC GAAATGGCGT CGACGGAGCG CGCAAAGCCA CTACTGCCAC

                                 esp3I
                                 bsmBI
                                 bsmAI
                              mspI                                 scrFI                        hgaI
                      fnu4HI  hpaII                                nciI                        thaI
                      bsoFI  scrFI                                 mspI                        fnuDII/mvnI
                      bbvI   nciI                                  hpaII                       bstUI  aciI
                   nlaIII    dsaV                              sfaNI                           bsh1236I
                  nspI       cauII            aluI            fokI dsaV                       hinPI nspBII
         mnlI     nspHI aluI bslI          maeIII           aciI   cauII     drdI             hhaI/cfoI         aciI
4301 AAAACCTCTG ACACATGCAG CTCCCGGAGA CGGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGCGCGTCAG CGGGTGTTGG
     TTTTGGAGAC TGTGTACGTC GAGGGCCTCT GCCAGTGTCG AACAGACATT CGCCTACGGC CCTCGTCTGT TCGGGCAGTC CCGCGCAGTC GCCCACAACC
```

FIG. 41O

```
                                                                                                            hgiAI/aspHI
          fnu4HI                                                                                            bsp1286
          bsoFI             maeII                                           sfaNI                           bsiHKAI
          bbvI            maeIII                                          fnu4HI                    ddeI    bmyI  ndeI
        hinPI  nlaIII bsrI bsaAI                     bst1107I   tru9I     bsoFI                  rsaI       apaLI/snoI
        hhaI/cfoI  tth111I/aspI            aciI      accI bsrI  mseI      aciI                   csp6I      alw44I/snoI
4401 CGGGTGTCGG GGCGCAGCCA TGACCCAGTC ACGTAGCGAT AGCGGAGTGT ATACTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC
     GCCCACAGCC CCGCGTCGGT ACTGGGTCAG TGCATCGCTA TCGCCTCACA TATGACCGAA TTGATACGCC GTAGTCTCGT CTAACATGAC TCTCACGTGG

                                                                 mboII
                                                                earI/ksp632I
                                                               sapI                         hinPI
                                                             hinPI                          hhaI/cfoI
                                                             hhaI/cfoI                    fnu4HI
                                                   sfaNI                            pleI  bsoFI     mcrI
        aciI          aciI     sfaNI              aciI       haeII     aciI  mnlI   hinfI bbvI      bsiEI
4501 ATATGCGGTG TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG
     TATACGCCAC ACTTTATGGC GTGTCTACGC ATTCCTCTTT TATGGCGTAG TCCGCGAGAA GGCGAAGGAG CGAGTGACTG AGCGACGCGA GCCAGCAAGC

        fnu4HI
        bsoFI
        aciI                                                                            nlaIII        bslI
     fnu4HI     aciI                                                                    nspI         cac8I
     bsoFI   bsrBI                                               tfiI                   nspHI       haeIII/palI
     bbvI  cac8I        aluI          aciI                       hinfI                  aflIII      haeI
4601 GCTGCGGCGA GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA
     CGACGCCGCT CGCCATAGTC GAGTGAGTTT CCGCCATTAT GCCAATAGGT GTCTTAGTCC CCTATTGCGT CCTTTCTTGT ACACTCGTTT TCCGGTCGTT

        scrFI          thaI
        mvaI                fnuDII/mvnI
        ecoRII         bstUI
        dsaV           bsh1236I
        bstNI   bslI       aciI
        apyI[dcm+]        fnu4HI                                                          hgaI
      haeIII/palI         bsoFI    cac8I                      aciI                        drdI
     haeI  nlaIV         haeIII/palI                  nlaIV                  sfaNI       taqI         mnlI
4701 AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC
     TTCCGGTCCT TGGCATTTTT CCGGCGCAAC GACCGCAAAA AGGTATCCGA GGCGGGGGGA CTGCTCGTAG TGTTTTTAGC TGCGAGTTCA GTCTCCACCG
```

FIG. 41P

```
                                   scrFI
                  scrFI    mvaI    .
                  mvaI             ecoRII
                  ecoRII   dsaV                                       bslI
                  dsaV     bstNI                      hinPI                    aciI     mspI
                  bstNI             apyI[dcm+]         bssSI                          fnu4HI     hpaII
                  apyI[dcm+]   bsaJI     aluI mnlI     hhaI/cfoI                      bsoFI     bsaWI        aciI
4801 GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC
     CTTTGGGCTG TCCTGATATT TCTATGGTCC GCAAAGGGGG ACCTTCGAGG GAGCACGCGA GAGGACAAGG CTGGGACGGC GAATGGCCTA TGGACAGGCG

                                                                                                      hgiAI/aspHI
                                                                                                      bsp1286
                                                                                                      bsiHKAI
                 hinPI                                                                                bmyI
                 hhaI/cfoI                                                                            apaLI/snoI
                haeII         aluI      scfI       ddeI                                     aluI      alw44I/snoI
4901 CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG TGTGCACGAA
     GAAAGAGGGA AGCCCTTCGC ACCGCGAAAG AGTATCGAGT GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT TCGACCCGAC ACACGTGCTT

                                                                                                   alwNI[dcm-]
              fnu4HI                                                  mspI                        fnu4HI
              bsoFI                                                   hpaII                       bsoFI
            nspBII                 maeIII                            scrFI                     fnu4HI
            aciI  hinPI        mspI                                  nciI                      bsoFI
         mcrI bbvI            bsaWI                    pleI          dsaV                      bbvI            maeIII
         bsiEI     hhaI/cfoI hpaII                     hinfI         cauII                 bsrI   bbvI   bsrI
5001 CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA
     GGGGGGCAAG TCGGGCTGGC GACGCGGAAT AGGCCATTGA TAGCAGAACT CAGGTTGGGC CATTCTGTGC TGAATAGCGG TGACCGTCGT CGGTGACCAT

                                                             bslI               rmaI
                                                           haeIII/palI          maeI                      hinPI
           mnlI        aciI    scfI                       haeI                  bfaI                      hhaI/cfoI
5101 ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC
     TGTCCTAATC GTCTCGCTCC ATACATCCGC CACGATGTCT CAAGAACTTC ACCACCGGAT TGATGCCGAT GTGATCTTCC TGTCATAAAC CATAGACGCG
```

FIG. 41Q

```
                                                  mspI
                                                   hpaII
                                                 sau3AI
                                                 mboI/ndeII[dam-]                                       fnu4HI
                                                 dpnI[dam+]                                             bsoFI
                  maeIII                         dpnII[dam-]         nspBII                              bbvI
                                          aluI   alwI[dam-]          aciI       aciI           cac8I
          eco57I bsrI
5201 TCTGCTGAAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG CAAGCAGCAG
     AGACGACTTC GGTCAATGGA AGCCTTTTTC TCAACCATCG AGAACTAGGC CGTTTGTTTG GTGGCGACCA TCGCCACCAA AAAAACAAAC GTTCGTCGTC

                                             sau3AI
                                       sau3AI mboI/ndeII[dam-]
                                       mboI/ndeII[dam-]
           hinPI          sau3AI   mboII[dam-] dpnI[dam+]
           hhaI/cfoI      mboI/ndeII[dam-]     dpnII[dam-]
          thaI            dpnI[dam+]   dpnI[dam+]                                                  tru9I          nlaIII
          fnuDII/mvnI     dpnII[dam-]  dpnII[dam-]                                                 mseI          rcaI
          bstUI          bstYI/xhoII   alwI[dam-]                                               maeII           bspHI
          bsh1236I       alwI[dam-]  bstYI/xhoII                      hgaI ddeI
5301 ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA
     TAATGCGCGT CTTTTTTTCC TAGAGTTCTT CTAGGAAACT AGAAAAGATG CCCCAGACTG CGAGTCACCT TGCTTTTGAG TGCAATTCCC TAAAACCAGT

                                   sau3AI
                                   mboI/ndeII[dam-]
                                 rmaI
                              hphI  dpnI[dam+]
                           mboII[dam-]
                          sau3AI    maeI     tru9I
                          mboI/ndeII[dam-]   mseI
                          dpnI[dam+]  dpnII[dam-]
                          dpnII[dam-] alwI[dam-]  tru9I          tru9I
                         bstYI/xhoII bstYI/xhoII  mseI           mseI
                         alwI[dam-] bfaI      ahaIII/draI       ahaIII/draI                              maeIII
5401 TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA
     ACTCTAATAG TTTTTCCTAG AAGTGGATCT AGGAAAATTT AATTTTTACT TCAAAATTTA GTTAGATTTC ATATATACTC ATTTGAACCA GACTGTCAAT

                           nlaIV          sau3AI
                           hgiCI          mboI/ndeII[dam-]              pleI
             tru9I         banI           dpnI[dam+]                    hinfI
             mseI         mnlI      ddeI  dpnII[dam-]      fokI         ahdI/eam1105I                   mnlI
5501 CCAATGCTTA ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC GATACGGGAG
     GGTTACGAAT TAGTCACTCC GTGGATAGAG TCGCTAGACA GATAAAGCAA GTAGGTATCA ACGGACTGAG GGGCAGCACA TCTATTGATG CTATGCCCTC
```

FIG. 41R

```
                                  bsmAI
                                  bsaI
           bsrI                 thaI                      bpmI/gsuI[dcm-]                 mspI
         sau96I    fnu4HI       fnuDII/mvnI          mspI                                 hpaII    haeIII/palI
         nlaIV     bsoFI        bstUI                hpaII                                bglI    sau96I hinPI
         haeIII/palI  bsrDI     bsh1236I             cfr10I/bsrFI                      cac8I      asuI   hhaI/cfoI
         asuI      bbvI       aciI              hphI  nlaIV
5601 GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC
     CCGAATGGTA GACCGGGGTC ACGACGTTAC TATGGCGCTC TGGGTGCGAG TGGCCGAGGT CTAAATAGTC GTTATTTGGT CGGTCGGCCT TCCCGGCTCG

                                                             scrFI
                                                             nciI
                                                             mspI                                          maeII
                                                             hpaII      rmaI                          hinPI
                                                 tru9I       dsaV       maeI                          hhaI/cfoI
      sau96I                                     mseI        cauII      bfaI                tru9I     mstI psp1406I
      avaII                   mnlI     bsrI      aseI/asnI/vspI    aluI             bsrI   mseI      aviII/fspI
      asuI                  aciI     fokI
5701 GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT
     CGTCTTCACC AGGACGTTGA AATAGGCGGA GGTAGGTCAG ATAATTAACA ACGGCCCTTC GATCTCATTC ATCAAGCGGT CAATTATCAA ACGCGTTGCA

            cac8I                                                                                  sau3AI
          scfI                                                                                     mboI/ndeII[dam-]
          pstI                                                       nlaIV      sau3AI             dpnI[dam+]
         fnu4HI                                                    mspI         mboI/ndeII[dam-]   dpnII[dam-]
         bsoFI                                                    bsaWI         dpnI[dam+]    nlaIII      nlaIII
         bbvI     mslI                                        aluI hpaII        dpnII[dam-] maeIII alwI[dam-]
     bsrDI bsgI  sfaNI       maeIII
5801 TGTTGCCATT GCTGCAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC ATGATCCCCC
     ACAACGGTAA CGACGTCCGT AGCACCACAG TGCGAGCAGC AAACCATACC GAAGTAAGTC GAGGCCAAGG GTTGCTAGTT CCGCTCAATG TACTAGGGGG

                              sau3AI
                              mboI/ndeII[dam-]                   aciI
                              dpnI[dam+]                         fnu4HI
                          mnlI dpnII[dam-]                       bsoFI                             fnu4HI
                        sau96I pvuI/bspCI                      haeIII/palI                         bsoFI
                        avaII   mcrI                           eaeI                    nlaIII      bbvI
              aciI   aluI   asuI    bsiEI                      cfrI                    mslI
5901 ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA
     TACAACACGT TTTTCGCCA ATCGAGGAAG CCAGGAGGCT AGCAACAGTC TTCATTCAAC CGGCGTCACA ATAGTGAGTA CCAATACCGT CGTGACGTAT
```

FIG. 41S

```
                                                                                                  mcrI
                                                                                                  bsiEI
                                                                                                  bcgI
                                                              rsaI                             fnu4HI
                                                     bsrI    scaI                              bsoFI
                      fokI                                                                     aciI
                 nlaIII          sfaNI        maeIII hphI  csp6I         ddeI
6001 ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC
     TAAGAGAATG ACAGTACGGT AGGCATTCTA CGAAAAGACA CTGACCACTC ATGAGTTGGT TCAGTAAGAC TCTTATCACA TACGCCGCTG GCTCAACGAG

       hgaI
      hinlI/acyI
      ahaII/bsaHI
    mspI                   hinPI                                                                           sau3AI
     hpaII                  hhaI/cfoI                                                                      mboI/ndeII[dam-]
    scrFI                  thaI                         hgiAI/aspHI                                        dpnI[dam+]
    nciI                   fnuDII/mvnI                  bsp1286          maeII                              dpnII[dam-]
    dsaV                   bstUI                 tru9I  bsiHKAI         psp1406I                           bstYI/xhoII
    cauII hincII/hindII    bsh1236I              mseI   bmyI           xmnI                                alwI[dam-]
                           aciI                ahaIII/draI             asp700   mboII
6101 TTGCCCGGCG TCAACACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT CTCAAGGATC
     AACGGGCCGC AGTTGTGCCC TATTATGGCG CGGTGTATCG TCTTGAAATT TTCACGAGTA GTAACCTTTT GCAAGAAGCC CCGCTTTTGA GAGTTCCTAG

                 bsrI                     hgiAI/aspHI
              sau3AI   taqI               bsp1286         eco57I
              mboI/ndeII[dam-]            bsiHKAI        mboII[dam-]
              dpnI[dam+]                  bmyI         sau3AI   sfaNI
              dpnII[dam-]                 apaLI/snoI   mboI/ndeII[dam-]
              alwI[dam-]                  alw44I/snoI  dpnI[dam+]
    nspBII                                             dpnII[dam-]
    aciI     bstYI/xhoII    maeIII     bssSI                                    hphI          hphI
6201 TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG
     AATGGCGACA ACTCTAGGTC AAGCTACATT GGGTGAGCAC GTGGGTTGAC TAGAAGTCGT AGAAAATGAA AGTGGTCGCA AAGACCCACT CGTTTTTGTC

             aciI
            fnu4HI                                           mboII
            bsoFI                                         earI/ksp632I   sspI
                                      mslI
6301 GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA
     CTTCCGTTTT ACGGCGTTTT TTCCCTTATT CCCGCTGTGC CTTTACAACT TATGAGTATG AGAAGGAAAA AGTTATAATA ACTTCGTAAA TAGTCCCAAT
```

FIG. 41T

```
                                                                                        hinPI
                                                                                      thaI
                                                                                      fnuDII/mvnI
                                                                                      bstUI                               maeII
      nlaIII                                                                          bsh1236I                            hinlI/acyI
     rcaI                                                                            aciI                                 ahaII/bsaHI
     bspHI  aciI                                                                 nlaIV  hhaI/cfoI                         aatII ddeI
  bsmAI  bsrBI
6401 TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG TGCCACCTGA CGTCTAAGAA
     AACAGAGTAC TCGCCTATGT ATAAACTTAC ATAAATCTTT TTATTTGTTT ATCCCCAAGG CGCGTGTAAA GGGGCTTTTC ACGGTGGACT GCAGATTCTT

                                                     sau96I
                                                     haeIII/palI
                                                     asuI       mboII
            nlaIII                                  eco0109I/draII
           rcaI    tru9I                           mnlI        bpuAI
           bspHI   mseI                         bssSI          bbsI
6501 ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTCTT CAA
     TGGTAATAAT AGTACTGTAA TTGGATATTT TTATCCGCAT AGTGCTCCGG GAAAGCAGAA GTT
```

FIG. 41U

```
>length: 6563

aatII(GACGTC):          1645 6489
acc65I(GGTACC):         403 823
accI(GTMKAC):           1093 1963 4449
accIII(TCCGGA):         3867[dam-]
aciI(CCGC):             178 542 805 877 1340 1750 1826 2011 2039 2043 2182 2242 2384 2492 2501 2504
                        2628 2781 2784 2787 2906 2926 3005 3045 3094 3141 3226 3241 3309 3342 3367 3412
                        3436 3448 3490 3544 3597 3613 3619 3700 3838 3967 3970 3981 4139 4155 4210 4266
                        4351 4390 4400 4442 4467 4505 4518 4544 4561 4604 4611 4632 4723 4751 4878 4897
                        5018 5128 5263 5272 5634 5725 5916 5962 6083 6127 6204 6313 6412 6459
acyI                    see hinlI
aflIII(ACRYGT):         1307 4678
ageI(ACCGGT):           1788
ahaII/bsaHI(GRCGYC):    1645 1813 2616 2637 2751 3408 6107 6489
ahaIII/draI(TTTAAA):    5435 5454 6146
ahdI/eam1105I(GACNNNNNGTC): 346 5566
aluI(AGCT):             72 121 252 320 398 532 589 648 1126 1144 1167 1325 1386 1906 2054 2075 2126
                        2218 2233 2889 3292 4202 4259 4270 4319 4338 4619 4845 4935 4981 5238 5759 5859
                        5922
alw44I/snoI(GTGCAC):    1831 4494 4992 6238
alwI[dam-](GGATC):      412 413 712 713 1171 1471 2578 2579 3300 3870 5245 5319 5331 5416 5429 5893
                        6196 6214
alwNI[dcm-](CAGNNNCTG): 1117 1385 5089
apaI(GGGCCC):           1695
apaLI/snoI(GTGCAC):     1831 4494 4992 6238
apoI(RAATTY):           1 391 4093
apyI[dcm+](CCWGG):      640 999 1347 1357 1449 1665 1713 1755 1764 2333 3262 3645 4705 4826 4839
aseI/asnI/vspI(ATTAAT): 5742
asnI                    see aseI
asp700(GAANNNNTTC):     905 930 4234 6166
asp718(GGTACC):         403 823
aspHI                   see hgiAI
aspI                    see tth111I
asuI(GGNCC):            1119 1195 1425 1434 1446 1512 1695 1696 1752 2155 2375 2727 3002 3090 3339 3463
```

FIG. 41V

Stop-Template Primer

SL.97.2 5' CAT GGT ATA GGT TAA ACT TAT TTA CAC 3'

NNS Randomization Primer

SL.97.3 5' CAT GGT ATA GGT NNS ACT TAT TTA CAC 3'

FIG. 42

Randomization of Position N35 of Variable Light Chain CDR-1
Amino Acid Frequency

Phage Display (NNS Codon Library) Sort #3

Amino Acid	Frequency	% Total	IC50 (nM)
Asparagine (wt)	1	5.6	4.9
Glycine	6	16.6	3.1
Aspartic Acid	3	16.6	3.1
Glutamic Acid	4	22.2	0.1
Alanine	2	5.6	0.2
Lysine	1	5.6	ND
Serine	1	1.9	ND

FIG. 43A

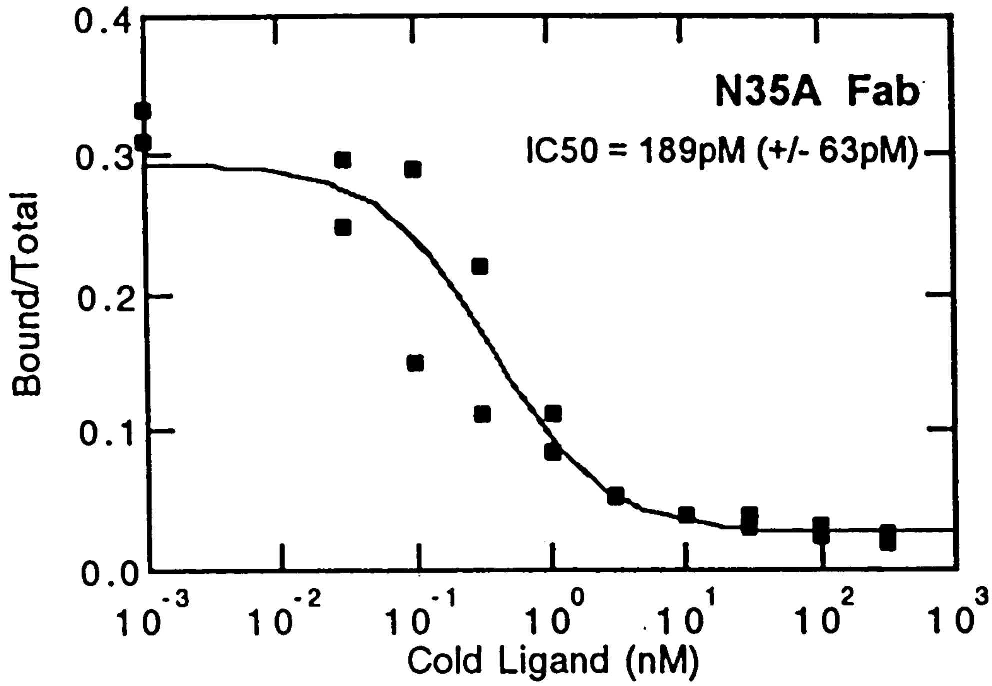


FIG. 43B-1

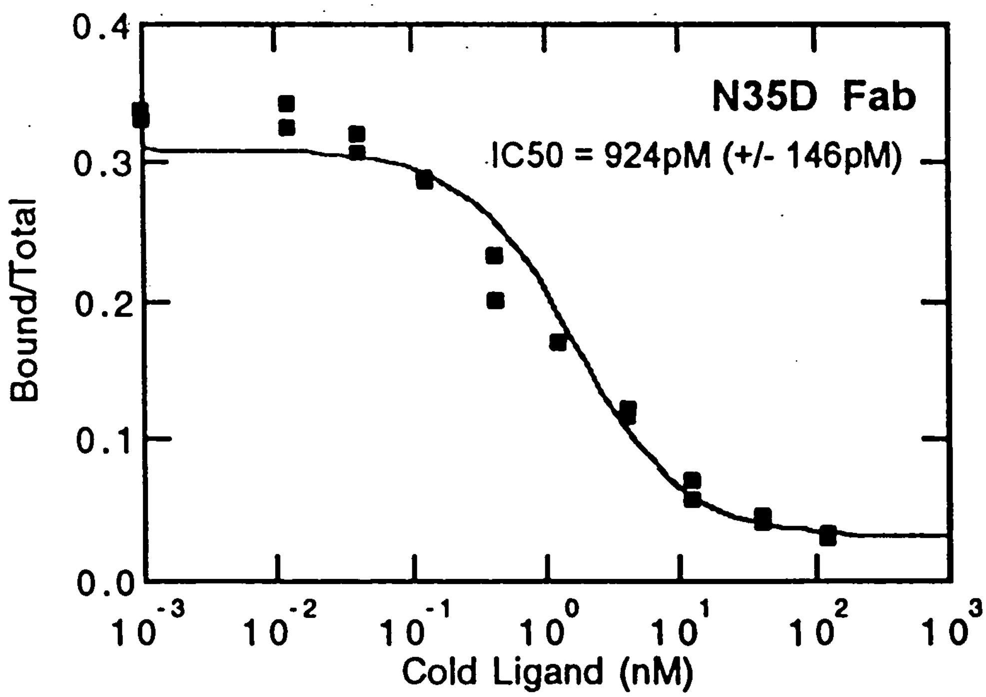


FIG. 43B-2

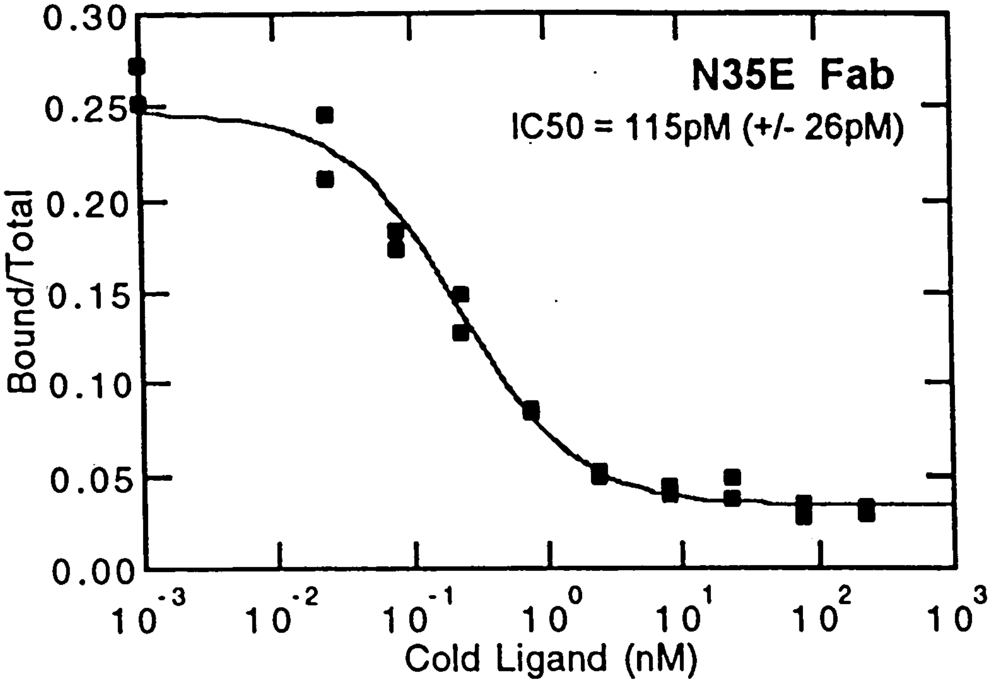


FIG. 43B-3

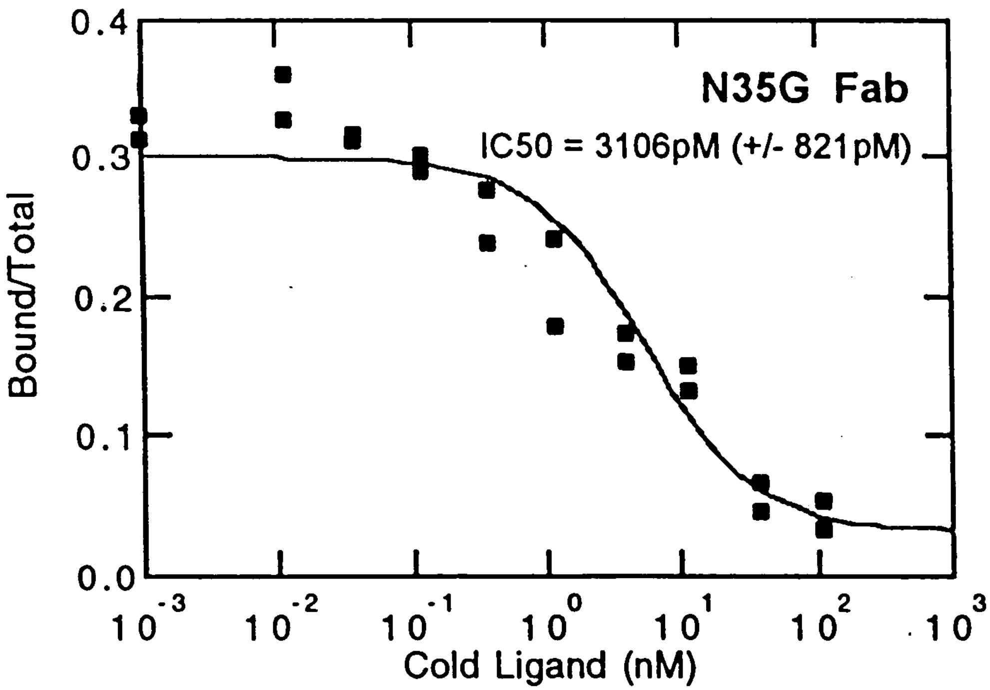
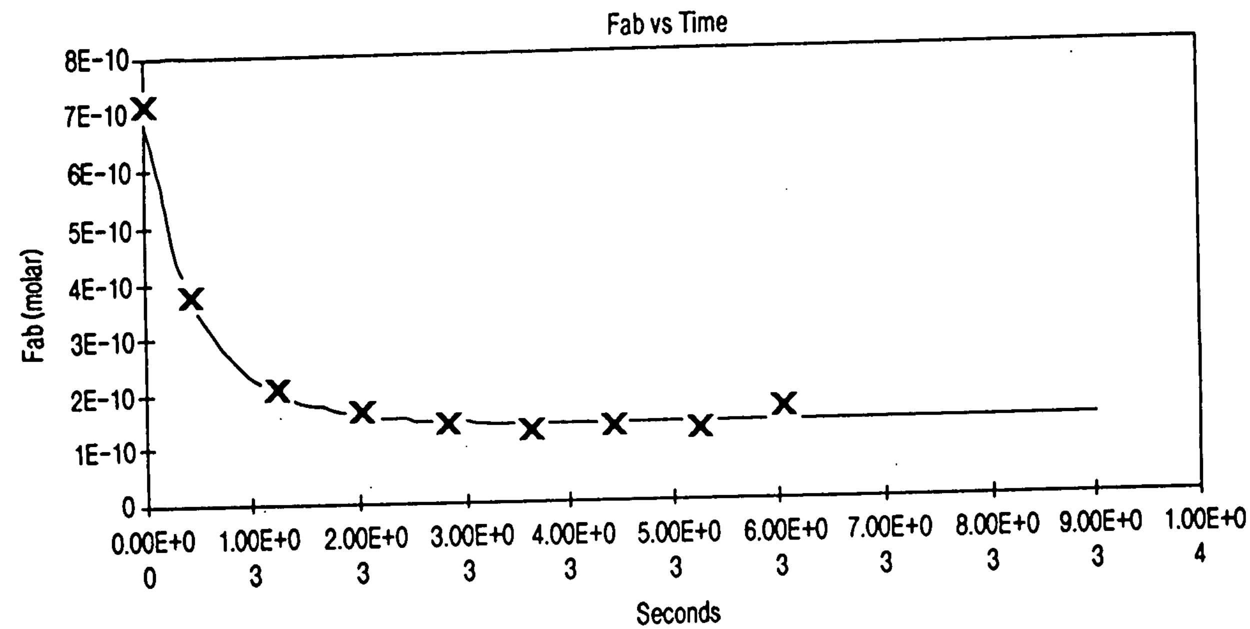


FIG. 43B-4



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.F(ab')2.

SAMPLE	ka	kd	Kd
6G4V11N35A-Fab	ND	ND	114pM
6G4V11N35A-F(ab')$_2$	2.0×10^6	2.1×10^{-4}	109pM
6G4V11N35E-Fab	4.7×10^6	2.6×10^{-4}	54pM

FIG. 44

```
1   ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

61  GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
-3  A  Y  A  D   I  Q  M   T  Q  S   P  S  S  L   S  A  S   V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGAGACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACTCTGCATA
18  R  V  T  I   T  C  R   S  S  Q   S  L  V  H   G  I  G   E  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
38  L  H  W  Y   Q  Q  K   P  G  K   A  P  K  L   L  I  Y   K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
58  N  R  F  S   G  V  P   S  R  F   S  G  S  G   S  G  T   D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
78  L  T  I  S   S  L  Q   P  E  D   F  A  T  Y   Y  C  S   Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
98  H  V  P  L   T  F  G   Q  G  T   K  V  E  I   K  R  T   V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P  S  V  F   I  F  P   P  S  D   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218 E  C  O
```

FIG. 45

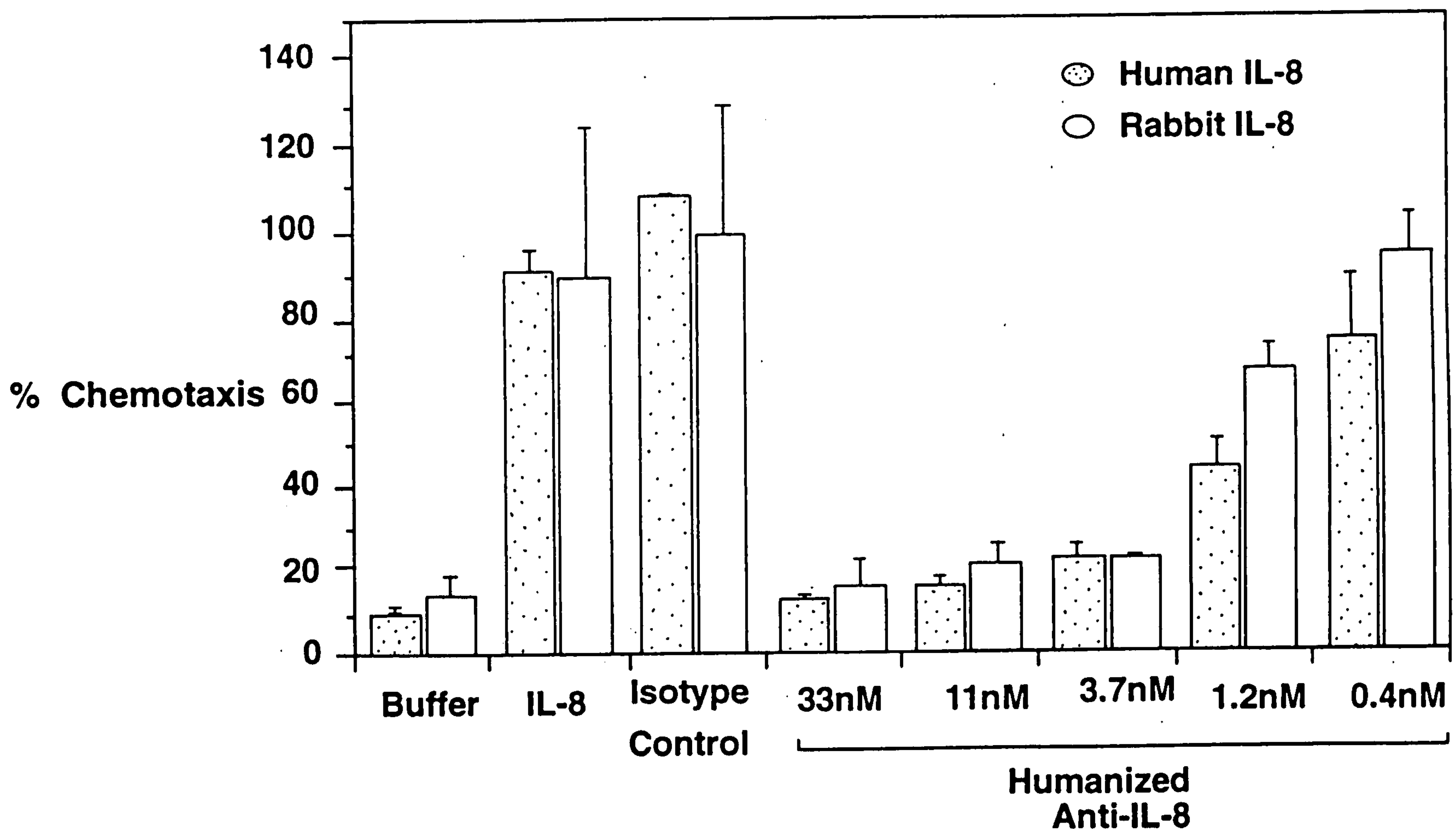

Human IL-8
Rabbit IL-8
% Chemotaxis
140
120
100
80
60
40
20
0
Buffer
IL-8
Isotype Control
33nM
11nM
3.7nM
1.2nM
0.4nM
Humanized Anti-IL-8

FIG. 46

N35AH1upr

5'-CTAGTGCAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCCTGTGCAGCTTCTGGCTACTCCTTC-3'

N35AH1lwr

5'-TCGAGAAGGAGTAGCCAGAAGCTGCACAGGACAAACGGAGTGAGCCCCCTGGCTGCACCAGGCCACCGCCAGACTGCACT
AG-3'

Bold indicates nucleotide change destroying PvuII site.

FIG. 47

```
> Wed May  7 18:27:36 1997
> /home/ruby/vc/Immbio/afan/ss.p6G425v11.N35A.choSD
> sites: std
> length: 8120 (circular)
>This has the pSVI backbone with the pRK7 cloning linker (pSVI7) and the intron DHFR(ID)
>made from pSVI.WTSD.D by adding a linearization linker(LL) into the HpaI site

      cac8I
     aluI
    sstI
    sacI
    hgiJII
    hgiAI/aspHI
    ecl136II
    bsp1286
    bsiHKAI
    bmyI
    banII
   taqI
 1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
   AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

   sau3AI  aluI
   mboI/ndeII[dam-]
   dpnI[dam+]
   pvuI/bspCI
   pleI dpnII[dam-]
   hinfI  taqI[dam-]
   rmaI  mcrI  pvuII
   maeI  bsiEI  nspBII
   bfaI  taqI[dam-]

   scrFI
   mvaI
   ecoRII
   dsaV
   bstNI
   apyI[dcm+]
   bsaJI
   bsmFI  nlaIV  cac8I

   sfaNI
   ppu10I
   nsiI/avaIII
   nlaIII
   sphI
   nspI
   nspHI
   cac8I

   scrFI
   mvaI
   ecoRII
   dsaV
   bstNI
   apyI[dcm+]
   sexAI

   scrFI
   mvaI  ecoRII
   dsaV
   bstNI
   apyI[dcm+]
   bsaJI
   bsmFI  nlaIV  cac8I

   sfaNI
   ppu10I
   nsiI/avaIII
   nlaIII
   sphI
   nspI
   nspHI
   cac8I
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

   aciI
   bsmFI
   aciI  fokI
   aciI
   aciI bsrI  aciI

   nlaIII
   styI
   ncoI
   bslI dsaI
   aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAATAA
```

FIG. 48A

```
                                                                                rmaI
                                                                                maeI
                                                                                styI                                haeIII/palI
          fnu4HI                                                                bsaJI                    aluI       mcrI
          bsoFI                                                                 blnI                    rmaI        eagI/xmaIII/eclXI
          bglI                                                                  avrII[dam-]             maeI        eaeI
         sfiI                                                                 haeIII/palI               bfaI        cfrI
         haeIII/palI                                                         stuI                      nheI         bsiEI
       mnlI    mnlI       ddeI                                 mnlI          haeI                      cac8I       mspI
 haeIII/palI bsaJI   mnlI    aluI                          mnlI             mnlI bfaI                 aluI        hpaII
     mnlI bsaJI aciI     haeIII/palI                       bseRI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC

                         tfiI
                         hinfI
    scrFI              aciI                                                                      fnu4HI
    nciI              thaI                                                                       bsoFI
    mspI              fnuDII/mvnI                                      aciI                      bbvI
    hpaII             bstUI                                 maeII  rsaI                         nspBII
    dsaV              bsh1236I                            maeIII   csp6I   scfI          mnlI    aciI      nlaIII
    cauII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA
                                                  ^splice donor                                     DHFR ATG^

                                                                                haeIII/palI
                                                                               haeI
                                                                              scrFI
                                                                              mvaI      bsrBI
                                                                              ecoRII
                                                                              dsaV
                                                                              bstNI     aciI                 rsaI
                               pflMI                                          apyI[dcm+]          xmnI       csp6I
                               bslI                            bsmAI
    taqI         sfaNI      bsmFI                              bsaI         bsaJI   mnlI  ddeI    asp700     scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG
```

FIG. 48B

```
                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
              eco57I                                                      bstNI                       tfiI        tru9I
             mboII                      tfiI                              apyI[dcm+]                  hinfI       mseI
            earI/ksp632I                hinfI  hphI                       sexAI               ddeI mboII taqI   ahaIII/draI
           mnlI                        alwNI[dcm-]
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                              sstI
                                                              sacI
                                                              hgiJII
                                                              hgiAI/aspHI
                                                              ecl136II
                                                              bsp1286
                                                              bsiHKAI
                                                              bmyI
        tru9I                                              mnlI aluI                                     tru9I
        mseI         ddeI                                bssSI  banII                                  aflII/bfrI
       aseI/asnI/vspI                                 bslI   bseRI          bstXI       fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT

                                                                                      haeIII/palI
                                                                                     haeI
                                                                    scrFI        scrFI
                                                                    mvaI              mvaI
                                                                    ecoRII       ecoRII
      mspI                                                          dsaV   tfiI  dsaV
      hpaII                                                         bstNI   nlaIII     bstNI     ddeI pleI
     bsaWI             accI nlaIII              mnlI                apyI[dcm+]  hinfI  apyI[dcm+]     hinfI
801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
    TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA
```

FIG. 48C

```
                                                                                                                      hgaI
                                                                                                                     hinlI/acyI
                                                                                                                     ahaII/bsaHI
                                                                                                                  scrFI
      nlaIII                                                                                                      mvaI     mnlI
    sau3AI                                                                                                        ecoRII
    mboI/ndeII[dam-]                                                                                              dsaV
    dpnI[dam+]                      maeII                                                                         bstNI    ecoNI
    dpnII[dam-]                   aflIII                                                                          apyI[dcm+]     mnlI
maeIII alwI[dam-]  apoI       maeIII                                                          mnlI               bsaJI      bslI ddeI
901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
    CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

    scrFI
    mvaI
    ecoRII
    dsaV
    bstNI
    apyI[dcm+]
  sau96I
  avaII                                                                          sfaNI
  asuI   mnlI       sfaNI                 accI      mboII                          mboII                        mnlI      aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                                  ^END DHFR

                                                   styI
                                                   bsaJI
                                                 sau3AI
                   nlaIII                        mboI/ndeII[dam-]
                  styI                           dpnI[dam+]                    aluI
                  ncoI                           dpnII[dam-]                  fnu4HI      tru9I
ppu10I            dsaI bsmFI                     alwI[dam-]                   bsoFI       mseI
nsiI/avaIII       bsaJI          cac8I           bstYI/xhoII                  bbvI       aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG

                                                                                 sau96I
                                                                                 avaII
                                                                                 asuI
                                                                               scrFI
                                                                               mvaI
                                                                               ecoRII
```

FIG. 48D

```
                                                                       dsaV                          ecoRI
                                                                       bstNI                          taqI    apoI
                                                                       apyI[dcm+]       mnlI        claI/bsp106
        maeIII                                                    bslI  bsaJI           bsaJI       bspDI[dam-]
        hphI    scfI       fokI
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA
                                                                  seq from pRK6G425VH: Cla-AvrII^

                                                                                                        scrFI
                                                                                                        mvaI   fnu4HI
      nlaIII                                                                                            ecoRII
     styI                                                                                               dsaV
     pflMI                                                                                              bstNI  bsoFI
     ncoI                                                                                  rmaI          apyI[dcm+]
     dsaI                             rmaI                   rsaI                          maeI                   bbvI
     bslI  fokI                       maeI            bpmI/gsuI[dcm-]                      bfaI        haeI
     bsaJI          nlaIII   fokI     bfaI          bsrI      csp6I               aluI          aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTAGTGCAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGATCACGTC AGACCGCCAC CGGACCACGT
   1                                                                 E  V  Q   L  V  Q   S  G  G  G   L  V  Q

                                                                                                scrFI
                                                                                                nciI
                                                                                                mspI
                                                                                                hpaII
                                                                                                dsaV
                                                                                                cauII
                                                                                               xmaI/pspAI
                                                                                               smaI
                                                                                               scrFI
                                                                                               nciI
                                                                                               dsaV          scrFI
                                                                                               cauII         mvaI
                                                                                               bslI          ecoRII
        hgiJII                                                                                 bsaJI         dsaV
        bsp1286                                                                           avaI          bstNI
        bmyI                                                                                   bsaJI          bslI
     scrFI                                                      pleI                                       apyI[dcm+]
     mvaI banII                                            hinfI             sau96I     sau96I           haeIII/palI
     ecoRII               aluI                                               avaII      nlaIV
     dsaV               alwNI[dcm-]                      taqI                       asuI         haeIII/palI  sau96I
     bstNI                  fnu4HI                          xhoI                    nlaIV        asuI bslI  eco0109I/draII
     bsaJI                  bsoFI                           paeR7I
     apyI[dcm+]             bbvI                            avaI   maeIII       bsrI          eco0109I/draII asuI
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGCTTCTGGC TACTCCTTCT CGAGTCACTA TATGCACTGG GTCCGTCAGG CCCCGGGTAA GGGCCTGGAA
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCGAAGACCG ATGAGGAAGA GCTCAGTGAT ATACGTGACC CAGGCAGTCC GGGGCCCATT CCCGGACCTT
  14  P  G  G   S  L  R  L   S  C  A   A  S  G   Y  S  F  S   S  H  Y   M  H  W   V  R  Q  A   P  G  K   G  L  E
```

FIG. 48E

```
                bslI                                                                            thaI
              sau3AI                                                                            fnuDII/mvnI
              mboI/ndeII[dam-]                                        haeIII/palI               bstUI
              dpnI[dam+]                    maeII                    sau96I                     bsh1236I
              dpnII[dam-]                   snaBI                    asuI                      nruI
              alwI[dam-]        hphI        bsaAI
1501 TGGGTTGGAT ATATTGATCC TTCCAATGGT GAAACTACGT ATAATCAAAA GTTCAAGGGC CGTTTCACTT TATCTCGCGA CAACTCCAAA AACACAGCAT
     ACCCAACCTA TATAACTAGG AAGGTTACCA CTTTGATGCA TATTAGTTTT CAAGTTCCCG GCAAAGTGAA ATAGAGCGCT GTTGAGGTTT TTGTGTCGTA
  47 W  V  G  Y   I  D  P   S  N  G   E  T  T  Y   N  Q  K   F  K  G   R  F  T  L   S  R  D   N  S  K   N  T  A  Y

                                                                                                        hinlI/acyI
                                                                                                        ahaII/bsaHI
      scfI                                                                                   bsrI       aatII
      pstI                                                                                 maeIII      taqI
      bsgI                cac8I    mnlI                                    mnlI            hphI      mboII   maeII
     bspMI             cac8I     ddeI drdI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTACTGT GCAAGAGGGG ATTATCGCTA CAATGGTGAC TGGTTCTTCG ACGTCTGGGG
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATGACA CGTTCTCCCC TAATAGCGAT GTTACCACTG ACCAAGAAGC TGCAGACCCC
  81  L  Q  M   N  S  L   R  A  E  D   T  A  V   Y  Y  C   A  R  G  D   Y  R  Y   N  G  D   W  F  F  D   V  W  G

                                           sau96I                                                                scrFI
                                          sau96I                                                                 mvaI
                                          nlaIV                                                                  ecoRII
                                          hgiJII                     nlaIV                                       dsaV
                   esp3I                  bsp1286                    hgiCI                                       bstNI
          scrFI                       bsp120I                     banI                                    bsaJI
          mvaI         bsmBI              bmyI                      scrFI                                  sau96I
          ecoRII                      banII                 mvaI                                           haeIII/palI
          dsaV                        asuI          mboII   ecoRII                                  asuI
          bstNI hphI       mnlI                 apaI         bpuAI    dsaV         hgiAI/aspHI       fnu4HI
          apyI[dcm+]       bsaJI             styI  haeIII/palI        bstNI      bseRI   bsp1286            bsoFI
        bsaJI maeIII     bseRI     mnlI      bsaJI asuI       bbsI    apyI[dcm+]  mnlI   bsiHKAI   bsp1286 aciI bsaJI
     nlaIV    bstEII bsmAI     haeIII/palI eco0109I/draII            bsaJI       mnlI    bmyI  mnlI  bmyI  nspBII apyI[dcm+]
1701 TCAAGGAACC CTGGTCACCG TCTCCTCGGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA CCTCTGGGGG CACAGCGGCC
     AGTTCCTTGG GACCAGTGGC AGAGGAGCCG GAGGTGGTTC CCGGGTAGCC AGAAGGGGGA CCGTGGGAGG AGGTTCTCGT GGAGACCCCC GTGTCGCCGG
 114  Q  G  T   L  V  T  V   S  S  A   S  T  K   G  P  S  V   F  P  L   A  P  S   S  K  S  T   S  G  G   T  A  A
```

FIG. 48F

```
          scrFI
          mvaI
          ecoRII
          ecoNI
          dsaV
          bstNI
          bslI
          apyI[dcm+]
      fnu4HI                                                                      hinPI
      bsoFI                                                              nlaIV                      hgiAI/aspHI
      bbvI                                      maeIII                         narI                       bsp1286
                                                   hphI                   kasI                     bsiHKAI
                                                  mspI                           hinlI/acyI            bmyI          mspI
                                                  hpaII                          hgiCI              cac8I             hpaII
                                                  cfr10I/bsrFI                   haeII           fnu4HI              scrFI
                                                  bsaWI                          banI            bsoFI               nciI
                                       bslI       ageI  tth111I/aspI             ahaII/bsaHI     aciI apaLI/snoI     dsaV
                                                                            ddeI hhaI/cfoI  nspBII alw44I/snoI  cauII          scfI
1801 CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTGCA CACCTTCCCG GCTGTCCTAC
     GACCCGACGG ACCAGTTCCT GATGAAGGGG CTTGGCCACT GCCACAGCAC CTTGAGTCCG CGGGACTGGT CGCCGCACGT GTGGAAGGGC CGACAGGATG
 147 L  G  C  L   V  K  D   Y  F  P   E  P  V  T   V  S  W   N  S  G   A  L  T  S   G  V  H   T  F  P   A  V  L  Q

                                                               fnu4HI
                                                               bsoFI       nlaIV
                                                             rmaI          hgiCI
   ddeI pleI                  fnu4HI            bsp1286 maeI         banI                        tfiI
   mnlI  hinfI                bsoFI                     bfaI   aluI bsp1286                      hinfI
   eco81I             mnlI    bbvI          maeIII                                          maeII
   bsu36I/mstII/sauI  ddeI                 hphI     bmyI mnlI  bbvI bmyI
1901 AGTCCTCAGG ACTCTACTCC CTCAGCAGCG TGGTGACTGT GCCCTCTAGC AGCTTGGGCA CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA
     TCAGGAGTCC TGAGATGAGG GAGTCGTCGC ACCACTGACA CGGGAGATCG TCGAACCCGT GGGTCTGGAT GTAGACGTTG CACTTAGTGT TCGGGTCGTT
 181  S  S  G   L  Y  S   L  S  S  V   V  T  V   P  S  S   S  L  G  T   Q  T  Y   I  C  N   V  N  H  K   P  S  N

                                                                                                ahdI/eam1105I
                                                                                               sau96I
                                                                                               avaII
                                                                                        scrFI
                                                                                        mvaI   asuI
                                                                                        ecoRII
                                                                                        dsaV
                           hgiJII                                                       bstNI  nlaIV        mboII
   styI                    bsp1286                         nlaIII                       bsaJI  bsmFI        bpuAI
   bsaJI                   bmyI                          nspI            bsp1286       apyI[dcm+]           bbsI mnlI
  mslI                     banII          maeIII         nspHI           bmyI alwNI[dcm-]
2001 CACCAAGGTG GACAAGAAAG TTGAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
     GTGGTTCCAC CTGTTCTTTC AACTCGGGTT TAGAACACTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT GGACTTGAGG ACCCCCCTGG CAGTCAGAAG
 214  T  K  V   D  K  K  V   E  P  K   S  C  D   K  T  H  T   C  P  P   C  P  A   P  E  L  L   G  G  P   S  V  F
```

FIG. 48G

```
                                                        sau96I
                                                        nlaIV
                                                     mspI
                                                     hpaII
                                                    scrFI
                                                    nciI
                                                    dsaV
                                                sau3AI   avaII              nlaIII
                                                mboI/ndeII[dam-]          nspI
                                            nlaIII    cauII        mnlI    nspHI
                                            rcaI dpnI[dam+]       ddeI    mslI                                      drdI    mnlI
                                         mnlI    dpnII[dam-]     eco81I maeIII                                  mboII   ddeI
     mboII              styI           mslI bspHI[dam-]        asuI bsu36I/mstII/sauI                           bpuAI  eco81I
     earI/ksp632I       bsaJI                                                                    maeII          bbsI   bsu36I/mstII/sauI
2101 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
     GAGAAGGGGG GTTTTGGGTT CCTGTGGGAG TACTAGAGGG CCTGGGGACT CCAGTGTACG CACCACCACC TGCACTCGGT GCTTCTGGGA CTCCAGTTCA
247  L  F  P  P   K  P  K   D  T  L   M  I  S  R   T  P  E   V  T  C   V  V  V  D   V  S  H   E  D  P   E  V  K  F

                                                               aciI
                                                               thaI
                                                               fnuDII/mvnI
                                                               bstUI
                                                               bsh1236I
                                                               sacII/sstII
                                                               nspBII
                                                               kspI
                                                               dsaI
                                                               bsaJI                              rsaI
          maeII                                                aciI                               csp6I
          rsaI                                                 fnu4HI   mnlI     rsaI           maeII                  hphI
          csp6I                                                bsoFI    bseRI    csp6I          bsaAI           hgaI  mnlI
     bsrI bsaAI                  mnlI
2201 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
     AGTTGACCAT GCACCTGCCG CACCTCCACG TATTACGGTT CTGTTTCGGC GCCCTCCTCG TCATGTTGTC GTGCATGGCA CACCAGTCGC AGGAGTGGCA
281   N  W  Y   V  D  G   V  E  V  H   N  A  K   T  K  P   R  E  E  Q   Y  N  S   T  Y  R   V  V  S  V   L  T  V

          scrFI
          mvaI
          ecoRII                                                                                                 fnu4HI
          dsaV                                                                                                          bsoFI
     ecoNI bstNI bsrI                       rsaI       bsmAI                                                            bbvI
     bslI  apyI[dcm+]                       csp6I      bsaI              mnlI               taqI
2301 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
     GGACGTGGTC CTGACCGACT TACCGTTCCT CATGTTCACG TTCCAGAGGT TGTTTCGGGA GGGTCGGGGG TAGCTCTTTT GGTAGAGGTT TCGGTTTCCC
314   L  H  Q   D  W  L  N   G  K  E   Y  K  C   K  V  S  N   K  A  L   P  A  P   I  E  K  T   I  S  K   A  K  G
```

FIG. 48H

```
                                                    scrFI
                                                    nciI
                                                    mspI
                                                    hpaII
                                                    dsaV
                                                    cauII
                                                   xmaI/pspAI
                                                   smaI
                                                   scrFI                               scrFI          scrFI
                                                   nciI                                mvaI                mvaI
                                                   dsaV                                ecoRII         ecoRII
                                                   cauII                               dsaV           dsaV
                                                 fokI                                  bstNI               bstNI
                               rsaI          bslI  bsaJI mboII                         apyI[dcm+]          apyI[dcm+]
                               csp6I                                                   sexAI          bspMI
         avaI               bsp1407I/bsrGI  bslI  avaI  earI/ksp632I
2401 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAAGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
     GTCGGGGCTC TTGGTGTCCA CATGTGGGAC GGGGGTAGGG CCCTTCTCTA CTGGTTCTTG GTCCAGTCGG ACTGGACGGA CCAGTTTCCG AAGATAGGGT
347  Q  P  R  E   P  Q  V   Y  T  L   P  P  S  R   E  E  M   T  K  N   Q  V  S  L   T  C  L   V  K  G   F  Y  P  S

                                          mspI
                                          hpaII
           dsaI                         fnu4HI                                           pleI                      mnlI
           bslI                         bsoFI                                 mnlI       hinfI    nlaIV  mboII     scfI cac8I
       mslI bsaJI              bsrDI    bbvI
2501 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG
     CGCTGTAGCG GCACCTCACC CTCTCGTTAC CCGTCGGCCT CTTGTTGATG TTCTGGTGCG GAGGGCACGA CCTGAGGCTG CCGAGGAAGA AGGAGATGTC
381   D  I  A   V  E  W   E  S  N  G   Q  P  E   N  N  Y   K  T  T  P   P  V  L   D  S  D   G  S  F  F   L  Y  S

                                                    mboII
                                                   bpuAI                      nlaIII
           dsaI                      fnu4HI      maeII                    ppu10I                                   sapI
       hphI                          bsoFI     xmnI bbsI                  nsiI/avaIII                              mboII mnlI
     aluI  bsaJI             bspMI   bbvI      asp700          nlaIII     sfaNI    mnlI                            earI/ksp632I
2601 CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
     GTTCGAGTGG CACCTGTTCT CGTCCACCGT CGTCCCCTTG CAGAAGAGTA CGAGGCACTA CGTACTCCGA GACGTGTTGG TGATGTGCGT CTTCTCGGAG
414  K  L  T   V  D  K  S   R  W  Q   Q  G  N   V  F  S  C   S  V  M   H  E  A   L  H  N  H   Y  T  Q   K  S  L
```

FIG. 48I

```
                                                                                  sau96I
                                                                          aciI    haeIII/palI
                                                                         fnu4HI   asuI
          scrFI                                    taqI                  bsoFI nlaIII
          nciI                                   pleI                   sfiI   styI
          mspI                                 rmaI salI    scfI       eaeI   ncoI                 aluI
          hpaII                                maeI hincII/hindII      cfrI   dsaI                fnu4HI
          dsaV                             sau96I  hinfI    pstI                                   bsoFI
      bsmAI                                haeIII/palI      bsgI    aluI haeIII/palI               bbvI        maeIII
   bslI   cauII                            asuI bfaI accI bspMI   hindIII bglI bsaJI
2701 TCCCTGTCTC CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTGGCCG CCATGGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA
     AGGGACAGAG GCCCATTTAC TCACGCTGCC GGGATCTCAG CTGGACGTCT TCGAACCGGC GGTACCGGGT TGAACAAATA ACGTCGAATA TTACCAATGT
447  S  L  S  P   G  K  O

                                                                 rmaI
                                                                 maeI                                   nlaIII alwI(dam-)
             sfaNI   apoI                                   bsmI bfaI
2801 AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG
     TTATTTCGTT ATCGTAGTGT TTAAAGTGTT TATTTCGTAA AAAAAGTGAC GTAAGATCAA CACCAAACAG GTTTGAGTAG TTACATAGAA TAGTACAGAC

        sau3AI
        mboI/ndeII(dam-)
        dpnI(dam+)
        dpnII(dam-)
       pvuI/bspCI
       mcrI
       bsiEI                                                                          rsaI
      taqI(dam-)  tru9I                                                               csp6I
     claI/bsp106(dam-)        fnu4HI   haeI                                          nlaIV
     bspDI(dam-)  mseI        bsoFI styI                                             kpnI
    sau3AI       xmnI         bbvI  ncoI                                             hgiCI
    mboI/ndeII(dam-)         hinPI  dsaI haeIII/palI                                 banI
    dpnI(dam+) asp700        hhaI/cfoI nlaIII                                        asp718     mnlI
    dpnII(dam-)  aseI/asnI/vspI   bsaJI            mnlI        mnlI                  acc65I  ddeI  aciI
2901 GATCGATCGG GAATTAATTC GGCGCAGCAC CATGGCCTGA AATAACCTCT GAAAGAGGAA CTTGGTTAGG TACCTTCTGA GGCGGAAAGA ACCATCTGTG
     CTAGCTAGCC CTTAATTAAG CCGCGTCGTG GTACCGGACT TTATTGGAGA CTTTCTCCTT GAACCAATCC ATGGAAGACT CCGCCTTTCT TGGTAGACAC
```

FIG. 48J

```
                                              scrFI                                             sfaNI
                                              mvaI                                                 ppu10I                         scrFI
                                              ecoRII                                            nsiI/avaIII                  mvaI
                                              dsaV                                             nlaIII                        ecoRII
                                              bstNI                                                sphI                            dsaV
                                              apyI[dcm+]                                           nspI                            bstNI
                                             bsaJI                                                 nspHI                           apyI[dcm+]
                                         bsmFI   nlaIV       cac8I                                 cac8I                          sexAI         bsmFI
3001 GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG
     CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC

       nlaIV                                              sfaNI
     scrFI                                     ppu10I
     mvaI                                            nsiI/avaIII
     ecoRII                                   nlaIII
     dsaV                                    sphI
     bstNI                                         nspI
     apyI[dcm+]                                    nspHI                                 aciI                   aciI
    bsaJI           cac8I                          cac8I                              bsmFI          aciI   fokI              aciI
3101 TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC
     AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG

                                                                                  fnu4HI
                                                                                  bsoFI
                                                                                  bglI
                                                                                 sfiI
                                    nlaIII                                       haeIII/palI
                                   styI                                        mnlI    mnlI        ddeI
                                   ncoI                                   haeIII/palI bsaJI  mnlI   aluI               mnlI
                               bslI dsaI                                mnlI bsaJI aciI    haeIII/palI                  bseRI
      bsrI   aciI              aciI bsaJI
3201 CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG
     GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC
```

FIG. 48K

```
                                                            scrFI
                                                            nciI
                                                            mspI
                         rmaI                               hpaII
                         maeI                               dsaV
                        styI                             haeIII/palI
                        bsaJI                  aluI     mcrI                            tfiI
                        blnI                 rmaI       eagI/xmaIII/eclXI               hinfI
                        avrII[dam-]          maeI       eaeI                         aciI
                      haeIII/palI            bfaI       cfrI                        thaI
                     stuI                   nheI        bsiEI                       fnuDII/mvnI
                     haeI                   cac8I      mspI cauII                   bstUI                pleI
       mnlI         mnlI bfaI              aluI        hpaII                        bsh1236I             hinfI
3301 AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTCAGGTAA
     TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCAGTCCATT
                        ^seq from pSVI6B5-6G4VL: AvrII - HindIII frag                 U1 matched splice donar^

                                                                                             sau3AI
                                                                                             mboI/ndeII[dam-]
                                                                                             dpnI[dam+]
                                                                                             dpnII[dam-]
                                                                                             alwI[dam-]
                                                                                          taqI[dam-]
                                                        fnu4HI                           claI/bsp106[dam-]
                                                        bsoFI                            bspDI[dam-]
                                                        aciI                            sau3AI
                               bstXI                   thaI                             mboI/ndeII[dam-]
        aciI          scfI  sau96I    styI             fnuDII/mvnI tru9I                dpnI[dam+]
     rsaI            pleI   haeIII/palI                bstUI       mseI                 dpnII[dam-]
     csp6I  scfI     hinfI  asuI      bsaJI            bsh1236I   aseI/asnI/vspI       alwI[dam-]             fokI
3401 GTACCGCCTA TAGAGTCTAT AGGCCCACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTTTGGATC GATCCTACTG ACACTGACAT
     CATGGCGGAT ATCTCAGATA TCCGGGTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAAAACCTAG CTAGGATGAC TGTGACTGTA
                                               ^sp6 promoter                         ^removed ATG
                                                                                                ^U2 match
                                                                                          lariat consensus^
                                                                                 IgG vH natural lariat restored^
```

FIG. 48L

```
                                          sau96I
                                          avaII
                                          asuI                                    rmaI
                                        scrFI                              maeI                              nlaIII
                                        mvaI                                      bfaI                            styI
                                        ecoRII                      thaI    nheI            claI/bsp106    pflMI
                                        dsaV                        fnuDII/mvnI          sfaNI          ncoI
                                        bstNI                           bstUI  cac8I      fnu4HI      ecoRI     dsaI
                                        apyI[dcm+]            mnlI      bsh1236I aluI     bsoFI taqI  apoI      bslI fokI
                                   bslI  bsaJI                bsaJI     nruI   aluI       bbvI bspDI[dam-]      bsaJI
3501 CCACTTTTTC TTTTTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCGCG AAGCTAGCTT GGGCTGCATC GATTGAATTC CACCATGGGA
     GGTGAAAAAG AAAAAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGCGC TTCGATCGAA CCCGACGTAG CTAACTTAAG GTGGTACCCT
                                                           ^cloning linker

                                                                                  aluI
                                                                                  sstI
                                                                                  sacI
                                                                                  hgiJII
                                                                                  hgiAI/aspHI
                                                                                  ecl136II
                                                                                  bsp1286
                                                                                  bsiHKAI
                                                                             bsmFI bmyI
                            rmaI                   rsaI                    bsrI   avaI                mnlI
                            maeI               bpmI/gsuI[dcm-]                                      aciI
        nlaIII   fokI       bfaI              bsrI   csp6I     ecoRV      tth111I/aspI banII
3601 TGGTCATGTA TCATCCTTTT TCTAGTAGCA ACTGCAACTG GAGTACATTC AGATATCCAG ATGACCCAGT CCCCGAGCTC CCTGTCCGCC TCTGTGGGCG
     ACCAGTACAT AGTAGGAAAA AGATCATCGT TGACGTTGAC CTCATGTAAG TCTATAGGTC TACTGGGTCA GGGGCTCGAG GGACAGGCGG AGACACCCGC
   1                                                        D  I  Q   M  T  Q  S   P  S  S   L  S  A   S  V  G  D

                   scfI                                                                      scrFI
                   pstI                                                                      mvaI
                   bsgI                          rsaI                                        ecoRII
        hphI      sse8387I                  ddeI                     maeII                   dsaV
       maeIII     bspMI                   aluI  csp6I                snaBI                   bstNI     aluI
      bstEII    hphI  bspMI             hindIII     nlaIII          bsaAI        bsrI         apyI[dcm+]
3701 ATAGGGTCAC CATCACCTGC AGGTCAAGTC AAAGCTTAGT ACATGGTATA GGTGCTACGT ATTTACACTG GTATCAACAG AAACCAGGAA AAGCTCCGAA
     TATCCCAGTG GTAGTGGACG TCCAGTTCAG TTTCGAATCA TGTACCATAT CCACGATGCA TAAATGTGAC CATAGTTGTC TTTGGTCCTT TTCGAGGCTT
  18   R  V  T   I  T  C   R  S  S  Q   S  L  V   H  G  I   G  A  T  Y   L  H  W   Y  Q  Q   K  P  G  K   A  P  K
```

FIG. 48M

```
                                                          mspI
                                                          hpaII
                                                          bslI
                                                         bsaWI
                                                       sau3AI
                                                       mboI/ndeII[dam-]
                                                       dpnI[dam+]
                                                       dpnII[dam-]
                          tfiI                         alwI[dam-]                                    fnu4HI
                          hinfI       bsmFI            nlaIV                                         bsoFI
                        taqI     bpmI/gsuI[dcm-]       bstYI/xhoII                                   bbvI
                       claI/bsp106  pleI               bamHI                                        scfI
                       bspDI[dam-]  hinfI              alwI[dam-]    bsmFI                          pstI
                                                                                                     bsgI
3801 ACTACTGATT TACAAAGTAT CCAATCGATT CTCTGGAGTC CCTTCTCGCT TCTCTGGATC CGGTTCTGGG ACGGATTTCA CTCTGACCAT CAGCAGTCTG
     TGATGACTAA ATGTTTCATA GGTTAGCTAA GAGACCTCAG GGAAGAGCGA AGAGACCTAG GCCAAGACCC TGCCTAAAGT GAGACTGGTA GTCGTCAGAC
  51 L  L  I   Y  K  V  S   N  R  F   S  G  V   P  S  R  F   S  G  S   G  S  G   T  D  F  T   L  T  I   S  S  L

                                                                    rsaI
                                                                    csp6I
                                                                   nlaIV
                                                                   kpnI styI
                                                  bsrBI            hgiCI               sau3AI
        mboII                            rsaI     aciI             banI bsaJI          mboI/ndeII[dam-] fnu4HI
        bpuAI                            csp6I  bsmFI              asp718              dpnI[dam+]       bsoFI
        bbsI                            scaI   nlaIII       maeII  acc65I              dpnII[dam-]      bbvI
3901 CAGCCAGAAG ACTTCGCAAC TTATTACTGT TCACAGAGTA CTCATGTCCC GCTCACGTTT GGACAGGGTA CCAAGGTGGA GATCAAACGA ACTGTGGCTG
     GTCGGTCTTC TGAAGCGTTG AATAATGACA AGTGTCTCAT GAGTACAGGG CGAGTGCAAA CCTGTCCCAT GGTTCCACCT CTAGTTTGCT TGACACCGAC
  84 Q  P  E  D   F  A  T   Y  Y  C   S  Q  S  T   H  V  P   L  T  F   G  Q  G  T   K  V  E   I  K  R   T  V  A  A

                                                                                              haeIII/palI
          mboII                                                                               haeI
         bpuAI                                        xmnI                  xmnI             mnlI
         bbsI     mboII aciI                          asp700        cac8I   asp700
4001 CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA ACTGCTTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
     GTGGTAGACA GAAGTAGAAG GGCGGTAGAC TACTCGTCAA CTTTAGACCT TGACGAAGAC AACACACGGA CGACTTATTG AAGATAGGGT CTCTCCGGTT
 118  P  S  V   F  I  F   P  P  S  D   E  Q  L   K  S  G   T  A  S  V   V  C  L   L  N  N   F  Y  P  R   E  A  K
```

FIG. 48N

```
                                                     scrFI
                                                     mvaI
                                                     ecoRII
                                                     dsaV                                                                  fnu4HI
                                                     bstNI                                                          ddeI   bsoFI
     rsaI                   mnlI                     apyI[dcm+]                                               scfI  mnlI   bbvI
     csp6I                  bslI          maeIII bsaJI        maeIII
4101 AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
     TCATGTCACC TTCCACCTAT TGCGGGAGGT TAGCCCATTG AGGGTCCTCT CACAGTGTCT CGTCCTGTCG TTCCTGTCGT GGATGTCGGA GTCGTCGTGG
151  V  Q  W   K  V  D  N   A  L  Q   S  G  N   S  Q  E  S   V  T  E   Q  D  S   K  D  S  T   Y  S  L   S  S  T

                                                                              sstI
                                                                              sacI
                                                                              hgiJII
                                                                              hgiAI/aspHI
                                                                              ecl136II
                                                                              bsp1286
                                                                              bsiHKAI
                                                                              bmyI
                                                                            ddeI cac8I
                                                                         haeIII/palI
        ddeI                                                            sau96I aluI
       celII/espI                                                       asuI  banII
       blpI/bpu1102I                                     hphI          eco0109I/draII
    hgaI                                accI    cac8I    maeIII        alwNI[dcm-]       maeIII    aluI
4201 CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
     GACTGCGACT CGTTTCGTCT GATGCTCTTT GTGTTTCAGA TGCGGACGCT TCAGTGGGTA GTCCCGGACT CGAGCGGGCA GTGTTTCTCG AAGTTGTCCC
184 L  T  L  S   K  A  D   Y  E  K   H  K  V  Y   A  C  E   V  T  H   Q  G  L  S   S  P  V   T  K  S   F  N  R  G

                       sau96I
                aciI   haeIII/palI
               fnu4HI  asuI
               bsoFI nlaIII
              sfiI  styI
          aluI haeIII/palI                          aluI
        hindIII bglI ncoI                          fnu4HI
       tru9I    eaeI  dsaI                         bsoFI
       mseI     cfrI  bsaJI                        bbvI         maeIII           sfaNI  apoI
4301 GAGAGTGTTA AGCTTGGCCG CCATGGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT
     CTCTCACAAT TCGAACCGGC GGTACCGGGT TGAACAAATA ACGTCGAATA TTACCAATGT TTATTTCGTT ATCGTAGTGT TTAAAGTGTT TATTTCGTAA
218   E  C  O
```

FIG. 48O

```
                                                                       sau3AI
                                                                       mboI/ndeII[dam-]
                                                                       dpnI[dam+]
                                                                       dpnII[dam-]
                                                                      pvuI/bspCI
                                                                      mcrI
                                                                      bsiEI
                                                                     taqI[dam-]
                                                                    claI/bsp106[dam-]
                                                                    bspDI[dam-]  tru9I       fnu4HI     haeI
                                                                   sau3AI        mseI        bsoFI styI
                                                                   mboI/ndeII[dam-]          bbvI  ncoI
                                                                   dpnI[dam+] xmnI          hinPI  dsaI haeIII/palI
                 rmaI                                              dpnII[dam-]  aseI/asnI/vspI     bsaJI
                 maeI                                        nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII
            bsmI bfaI
4401 TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG GATCGATCGG GAATTAATTC GGCGCAGCAC CATGGCCTGA
     AAAAAGTGAC GTAAGATCAA CACCAAACAG GTTTGAGTAG TTACATAGAA TAGTACAGAC CTAGCTAGCC CTTAATTAAG CCGCGTCGTG GTACCGGACT
                                                                                ^sv40 origin

                                                                                                     scrFI
                                  rsaI                                                               mvaI
                                  csp6I                                                              ecoRII
                                 nlaIV                                                               dsaV
                                 kpnI                                                                bstNI
                                 hgiCI                                                               apyI[dcm+]
                                 banI                     aluI                                      bsaJI
                                 asp718     mnlI          pvuII                                  bsmFI   nlaIV
       mnlI       mnlI           acc65I    ddeI  aciI     nspBII
4501 AATAACCTCT GAAAGAGGAA CTTGGTTAGG TACCTTCTGA GGCGGAAAGA ACCAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC
     TTATTGGAGA CTTTCTCCTT GAACCAATCC ATGGAAGACT CCGCCTTTCT TGGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG

                              sfaNI                                            scrFI                           sfaNI
                            ppu10I                      scrFI         mvaI                                    ppu10I
                            nsiI/avaIII                 mvaI                   ecoRII                         nsiI/avaIII
                           nlaIII                       ecoRII                                          nlaIII
                          sphI                          dsaV          dsaV                                  sphI
                          nspI                          bstNI         bstNI                                       nspI
                          nspHI                         apyI[dcm+]             apyI[dcm+]                         nspHI
                          cac8I                         sexAI                 bsaJI                               cac8I
       cac8I                                                             bsmFI      nlaIV     cac8I
4601 CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG
```

FIG. 48P

```
                                                                                                  nlaIII
                                                                                                 styI
                                                                                                 ncoI
                                                                                            bslI dsaI
       aciI                                   aciI                                          aciI bsaJI
     bsmFI                      aciI   fokI                      aciI bsrI   aciI
4701 ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT
     TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA

                                                                                    rmaI
                                                                                    maeI
         fnu4HI                                                                    styI
         bsoFI                                                                     bsaJI
         bglI                                                                      blnI
        sfiI                                                                       avrII(dam-)
        haeIII/palI                                                              haeIII/palI
      mnlI   mnlI      ddeI                                 mnlI                stuI
  haeIII/palI bsaJI  mnlI    aluI                       mnlI                    haeI                  maeIII
mnlI bsaJI aciI    haeIII/palI                          bseRI                 mnlI bfaI             aluI
4801 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTG
     AAAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAC
                                                                                              start pUC118^

        fnu4HI
       haeIII/palI       hinPI
      mcrI               hhaI/cfoI
      eagI/xmaIII/eclXI thaI
      eaeI              fnuDII/mvnI
    notI                bstUI
  bsrBI bsoFI          hinPI             bspMI
 taqI  cfrI            hhaI/cfoI       scfI                                                                   scrFI
xhoI fnu4HI   tru9I    cac8I     tru9I pstI               haeIII/palI                                         mvaI
paeR7I bsiEI  pacI    ascI     ahaIII/draI               eaeI                                                 ecoRII
avaI bsoFI    mseI tru9I bsh1236I mseI bsgI  maeIII      cfrI                      bsrI                       dsaV
mnlI  aciI aciI   mseI bssHII  swaI   sse8387I   aluI   bsrI              maeII maeIII                        bstNI
                                                                                                              apyI(dcm+)
                                                                                                             bsaJI
4901 TTACCTCGAG CGGCCGCTTA ATTAAGGCGC GCCATTTAAA TCCTGCAGGT AACAGCTTGG CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
     AATGGAGCTC GCCGGCGAAT TAATTCCGCG CGGTAAATTT AGGACGTCCA TTGTCGAACC GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG
       ^linearization linker inserted into HpaI site
```

FIG. 48Q

```
                                                                                      sau3AI
                                                                                      mboI/ndeII[dam-]
                                                                             sau96I   dpnI[dam+]
                                                     cac8I                   haeIII/palI
                                                    aluI                     asuI     dpnII[dam-]
                           fnu4HI                  pvuII                   mnlI aciI  pvuI/bspCI
                tru9I      bsoFI                   nspBII                mboII cac8I  mcrI
     maeIII     mseI       bbvI     fokI         cac8I                   earI/ksp632I bsiEI
5001 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT
     ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA

                 hinPI
                 hhaI/cfoI
                nlaIV
                narI                                                                                 hinPI
                kasI                                                                                thaI
                hinlI/acyI                                                                          fnuDII/mvnI
                hgiCI                                                                               bstUI scfI
                haeII     aciI                                                                      bsh1236I
                banI     sfaNI                                                                   rsaI hhaI/cfoI
   bglI         ahaII/bsaHI                     sfaNI    aciI         aciI   maeII               csp6I   bslI
5101 AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG
     TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC

                  aciI
                fnu4HI                fnu4HI
                bsoFI                 bsoFI
               thaI                  hinPI                                   hinPI
               fnuDII/mvnI           hhaI/cfoI                               hhaI/cfoI
      hinPI    bstUI                thaI                                  rmaI
      hhaI/cfoI hinPI               fnuDII/mvnI                       hinPI  haeII
   fnu4HI      hhaI/cfoI            bstUI                             hhaI/cfoI    bsrBI
   bsoFI    tru9I aciI              bsh1236I     aciI                haeII maeI    aciI
   aciI     mseI bsh1236I        maeIII bbvI  maeIII           cac8I      bfaI   cac8I          mboII
5201 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC
     ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG
```

FIG. 48R

```
                 mspI                          nlaIV
                  hpaII                        hgiJII
                 naeI                          bsp1286
                 cfr10I/bsrFI                  bmyI                                  nlaIV
             maeII cac8I            aluI       banII        nlaIV                    hgiCI  taqI
                                                                                     banI mnlI
5301 TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC
     AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG

                 maeII    haeIII/palI                        maeII pleI          tru9I     pleI
                draIII    sau96I                             drdI  hinfI  maeII  mseI      hinfI
         hphI   bsaAI     asuI
5401 TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT
     AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA

                                                                                 tru9I
                  bslI                                                           mseI             tru9I
      bsrI      bslI  avaI                                             haeIII/palI           aluI   mseI
5501 CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA
     GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT

                                                       hgiAI/aspHI
             thaI                                      bsp1286
             fnuDII/mvnI               maeII           bsiHKAI                    aciI
        tru9I  apoI tru9I             psp1406I         bmyI   ddeI                fnu4HI
        mseI bstUI   mseI            tru9I             apaLI/snoI  rsaI           bsoFI     tru9I
    apoI    bsh1236I        sspI mseI                  alw44I/snoI csp6I        sfaNI       mseI        aciI
5601 CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC
     GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG

                                                              hinPI                            sfaNI
                                                              hhaI/cfoI                      mspI
                        hinPI                                 thaI                           hpaII
                      fnu4HI                                  fnuDII/mvnI                    scrFI
           maeIII     bsoFI                                   bstUI                          nciI
         maeII bsrI  nlaIII hhaI/cfoI                      nspBII bsh1236I                   dsaV fokI
        bsaAI tth111I/aspI bbvI               aciI         aciI hgaI      drdI               cauII   aciI
5701 GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA
     CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT
```

FIG. 48S

```
                                                                   thaI
                 scrFI                                             fnuDII/mvnI
                 nciI                                              bstUI
                 mspI                                              bsh1236I
                 hpaII     nspI                                   hinPI
                 dsaV      nspHI                                  hhaI/cfoI                          mnlI
            esp3I       fnu4HI                                   thaI mnlI                        haeIII/palI
            bsmBI       bsoFI                                    fnuDII/mvnI          mboII     sau96I
       maeIII bsmAI     bbvI                                     bstUI                bpuAI     asuI bssSI
   aluI     bslI  cauII aluI nlaIII    mnlI     hphI      hphI   bsh1236I             bbsI     eco0109I/draII
5801 CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA
     GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT

                                                                                  nlaIV
                                                                                aciI
                                                                               thaI
                                                                               fnuDII/mvnI
                                                      hinlI/acyI               bstUI
                                                      ahaII/bsaHI              bsh1236I
                            nlaIII                    aatII                   hinPI
                      tru9I  rcaI                                             hhaI/cfoI
                      mseI   bspHI               ddeI maeII
5901 TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT
     ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA

                                rcaI
                               bspHI
                             bsrBI   bsmAI                                      mboII
                             aciI   nlaIII                         sspI         earI/ksp632I       mslI
6001 TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC
     AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG

                                                                                                     hgiAI/aspHI
                                                                                                     bsp1286
                                                                                          sau3AI     bsiHKAI
                                                                                          mboI/ndeII[dam-]
                                                                                          dpnI[dam+] bmyI
                                                                                          dpnII[dam-]
                             fnu4HI                                                   eco57I         apaLI/snoI
                             bsoFI                                hphI        hphI   sfaNI mboII[dam-] alw44I/snoI
                             aciI
6101 CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG
     GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC
```

FIG. 48T

```
                     sau3AI    nspBII     sau3AI
                     mboI/ndeII[dam-]     mboI/ndeII[dam-]                  maeII
                     dpnI[dam+]           dpnI[dam+]                       psp1406I          hgiAI/aspHI
                    bstYI/xhoII           dpnII[dam-]                      xmnI              bsp1286  tru9I
                bsrI dpnII[dam-]          alwI[dam-]                       asp700            bsiHKAI  mseI
     bssSI    maeIII taqI   alwI[dam-]  aciI  bstYI/xhoII              mboII                 bmyI    ahaIII/draI
6201 CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT
     GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA

                                  scrFI
          aciI                    nciI
         thaI                     mspI
         fnuDII/mvnI              hpaII
         bstUI                    dsaV
         bsh1236I              hinlI/acyI                    aciI                              rsaI
        hinPI                  hgaI cauII           mcrI  fnu4HI                               csp6I   bsrI
        hhaI/cfoI              ahaII/bsaHI     bcgI  bsiEI bsoFI          ddeI                scaI  hphI  maeIII
6301 GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC
     CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG

                                                                                                       sau3AI
                                                                              haeIII/palI              mboI/ndeII[dam-]
                                                                             eaeI                      dpnI[dam+]
                                                                             cfrI                      dpnII[dam-]
                                                   fnu4HI                   fnu4HI                    pvuI/bspCI
                                                   bsoFI                    bsoFI                     mcrI
          sfaNI      fokI   nlaIII                 bbvI  mslI   nlaIII      aciI                      bsiEI
6401 ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA
     TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT

                                                   nlaIII
                                                 sau3AI maeIII                        mspI
        sau96I                                   mboI/ndeII[dam-]     sau3AI   nlaIV
        avaII                                    dpnI[dam+]           mboI/ndeII[dam-] aluI
        asuI                                     dpnII[dam-]          dpnI[dam+]   hpaII
      mnlI         aluI   aciI            nlaIII alwI[dam-]           dpnII[dam-] bsaWI
6501 TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA
     AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT
```

FIG. 48U

```
                                                    hinPI                                                       mspI
                                                    hhaI/cfoI                                                   hpaII
                                  fnu4HI           mstI                                                aluI    scrFI
                                  bsoFI            aviII/fspI        bsrI                         rmaI    nciI        tru9I
                  mslI        cac8I  bsrDI      maeII            tru9I                           maeI    dsaV        mseI
             maeIII      sfaNI   bbvI        psp1406I            mseI                            bfaI    cauII      aseI/asnI/vspI
6601 CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA
     GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT

                                                    bglI                                                        mspI
                                                    sau96I      cac8I                                           hpaII
                                                    haeIII/palI                                                 cfr10I/bsrFI
                            sau96I                                                                          nlaIV hphI        bsmAI
        fokI      aciI      avaII        hinPI      asuI       mspI                                    bpmI/gsuI[dcm-]     bsaI
     bsrI    mnlI           asuI         hhaI/cfoI             hpaII
6701 GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT
     CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA

       aciI
      thaI          fnu4HI     haeIII/palI
      fnuDII/mvnI   bsoFI     sau96I                                                  pleI
      bstUI         bbvI     nlaIV                                                    hinfI
      bsh1236I bsrDI     bsrI asuI                  mnlI                         ahdI/eam1105I       fokI
6801 CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG
     GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC

         ddeI
      sau3AI        nlaIV
      mboI/ndeII[dam-]                                                                          tru9I
      dpnI[dam+]    hgiCI        tru9I                                                          mseI            tru9I
      dpnII[dam-]   banI mnlI    mseI         maeIII                                            ahaIII/draI     mseI
6901 ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA
     TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT

              rmaI
              maeI       sau3AI
          sau3AI hphI    mboI/ndeII[dam-]
          mboI/ndeII[dam-]
          dpnI[dam+]     dpnI[dam+]
          dpnII[dam-]    dpnII[dam-]                                                                                 sau3AI
      tru9I bstYI/xhoII  alwI[dam-]        nlaIII            maeII                                                   mboI/ndeII[dam-]
      mseI  alwI[dam-]  bstYI/xhoII        rcaI           tru9I                          hgaI                        dpnI[dam+]
     ahaIII/draI bfaI  mboII[dam-]         bspHI          mseI                     ddeI                              dpnII[dam-]
7001 TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA
     AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT
```

FIG. 48V

```
                  sau3AI                                                                                     sau3AI
         mboII[dam-]                                                                                         mboI/ndeII[dam-]
        sau3AI    mboI/ndeII[dam-] thaI                                                                      dpnI[dam+]
        mboI/ndeII[dam-]            fnuDII/mvnI                                                              dpnII[dam-]
        dpnI[dam+]  dpnI[dam+]      bstUI       cac8I                                                       alwI[dam-]
        dpnII[dam-] dpnII[dam-]     bsh1236I   fnu4HI                                      aciI          mspI
       bstYI/xhoII  alwI[dam-]     hinPI      bsoFI                                                     hpaII      aluI
       alwI[dam-]  bstYI/xhoII     hhaI/cfoI  bbvI                             aciI       nspBII
7101 TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA
     AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT

                                                                      rmaI
                          bsrI                hinPI                   maeI                    haeIII/palI
                        maeIII     eco57I     hhaI/cfoI               bfaI       bslI        haeI
7201 GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC
     CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG

                                                        fnu4HI
                                                        bsoFI                   scrFI
                                                        bbvI                    nciI
                                                     fnu4HI                     mspI
                                                 alwNI[dcm-]                    hpaII
                                                bsrI  bsoFI                     dsaV      pleI
  scfI     aciI        mnlI              maeIII     bbvI     bsrI                cauII     hinfI
7301 TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT
     AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA

                  aciI
                 nspBII                      hgiAI/aspHI
                fnu4HI                       bsp1286
    mspI        bsoFI                        bsiHKAI
    hpaII       bbvI mcrI                    bmyI
   bsaWI      hinPI  bsiEI                   apaLI/snoI
 maeIII       hhaI/cfoI                      alw44I/snoI   aluI                            ddeI       scfI
7401 AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG
     TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC
```

FIG. 48W

```
                                                                                                      scrFI
                                                                                                      mvaI
                                                                                                      ecoRII
                                                          mspI                                        dsaV
                                                          hpaII  fnu4HI                    bssSI      bstNI
           hinPI                                          bslI   bsoFI                   hinPI mnlI   bsaJI
           hhaI/cfoI                            aciI     bsaWI   aciI                    hhaI/cfoI   aluI apyI[dcm+]
           haeII
7501 TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA
     ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT

           scrFI
           mvaI
           ecoRII
           dsaV                                                                                 nlaIV
           bstNI                                                    taqI                        aciI
           apyI[dcm+]                              mnlI drdI     hgaI              sfaNI
7601 GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
     CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT

             haeIII/palI          haeIII/palI
            fnu4HI              scrFI
            bsoFI               mvaI bslI
            aciI                ecoRII
           thaI bslI            dsaV                      nlaIII
           fnuDII/mvnI          bstNI              haeIII/palI  nspI
           bstUI                apyI[dcm+]         haeI         nspHI                       tfiI
      cac8I  bsh1236I     nlaIV  haeI            cac8I          aflIII                      hinfI
7701 AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG
     TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC

                                  fnu4HI
                                  bsoFI              fnu4HI
                                  bbvI               bsoFI                        sapI  hinPI
                             cac8I aciI              bbvI pleI                    mboII hhaI/cfoI
                            bsrBI  fnu4HI    mcrI    hinPI  hinfI                 earI/ksp632I
      aciI          aluI    aciI   bsoFI     bsiEI   hhaI/cfoI              mnlI  aciI   haeII
7801 TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA
     ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT
```

FIG. 48X

```
                     thaI
                     fnuDII/mvnI
                     bstUI
                     bsh1236I
                    hinPI
                    hhaI/cfoI
                   thaI
                   fnuDII/mvnI                cac8I
                   bstUI    haeIII/palI      aluI
                   bsh1236I           tru9I  pvuII                                                        tru9I
   mnlI           bslI     eaeI  tfiI aseI/asnI/vspI                            cac8I       hinPI       mseI       maeIII
  aciI            aciI     cfrI  hinfI mseI  nspBII                      bsrI    aciI        hhaI/cfoI  aseI/asnI/vspI
7901 CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT
     GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA

                           scrFI
                           mvaI
                           ecoRII
                           dsaV
                     nlaIV bstNI
                     hgiCI apyI[dcm+]                   mspI                       aciI
   mnlI              banI bsaJI                         hpaII                     bsrBI                         aluI
8001 ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA
     TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT
```

FIG. 48Y

```
                   tru9I
                   mseI
                  aseI/asnI/vspI
                xmnI
         nlaIII   asp700
  8101 TGACCATGAT TACGAATTAA
       ACTGGTACTA ATGCTTAATT

>length: 8120

aatII(GACGTC):          1690 5947
acc65I(GGTACC):         2969 3967 4529
accI(GTMKAC):           823 1039 2738 4237
aciI(CCGC):             217 229 238 250 260 271 317 422 454 485 574 1385 1795 1871 2248 2250 2758 2982
                        3167 3179 3188 3200 3210 3221 3267 3372 3404 3449 3686 3949 4021 4318 4542 4727
                        4739 4748 4760 4770 4781 4827 4910 4914 5070 5127 5153 5166 5203 5217 5220 5248
                        5275 5680 5699 5741 5751 5790 5979 6026 6125 6234 6311 6355 6476 6522 6713 6804
                        7166 7175 7310 7420 7541 7560 7687 7715 7806 7827 7834 7877 7901 7911 7967 8070
acyI                    see hinlI
aflII/bfrI(CTTAAG):     786
aflIII(ACRYGT):         932 7758
ageI(ACCGGT):           1833
ahaII/bsaHI(GRCGYC):    988 1690 1858 5117 5947 6329
ahaIII/draI(TTTAAA):    696 4935 6290 6982 7001
ahdI/eam1105I(GACNNNNNGTC): 2087 6865
aluI(AGCT):             5 44 332 386 390 753 1097 1165 1370 1431 1951 2603 2751 2784 3282 3336 3340
                        3562 3566 3676 3733 3792 4270 4288 4311 4344 4554 4842 4896 4954 5047 5333 5590
                        5803 5822 6516 6579 6679 7200 7457 7593 7819 7937 8096
alw44I/snoI(GTGCAC):    1876 5651 6198 7444
```

FIG. 48Z

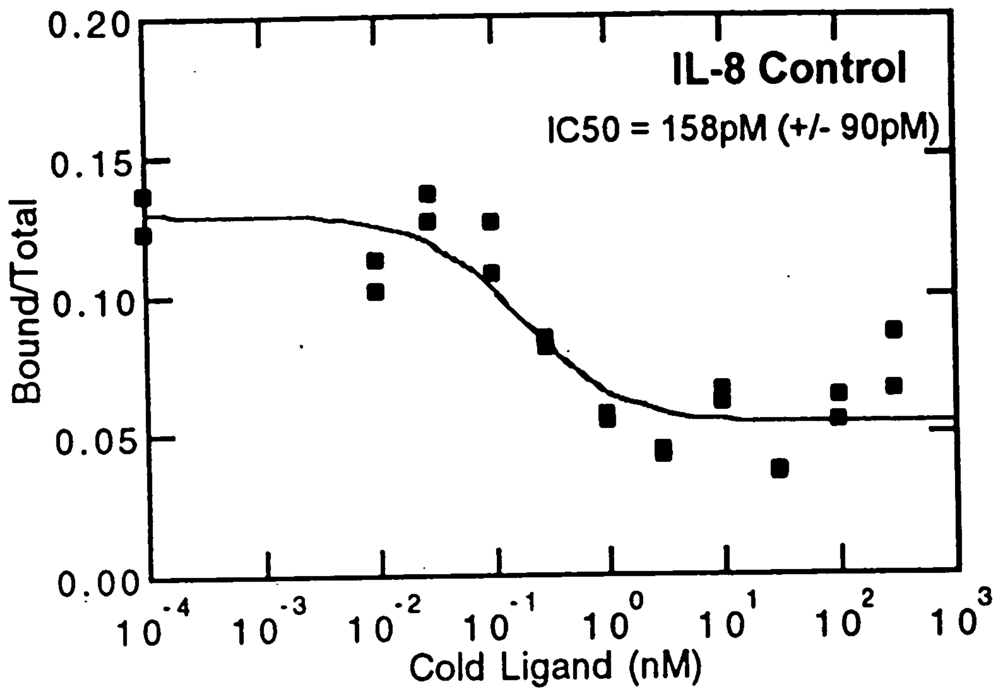


FIG. 49A

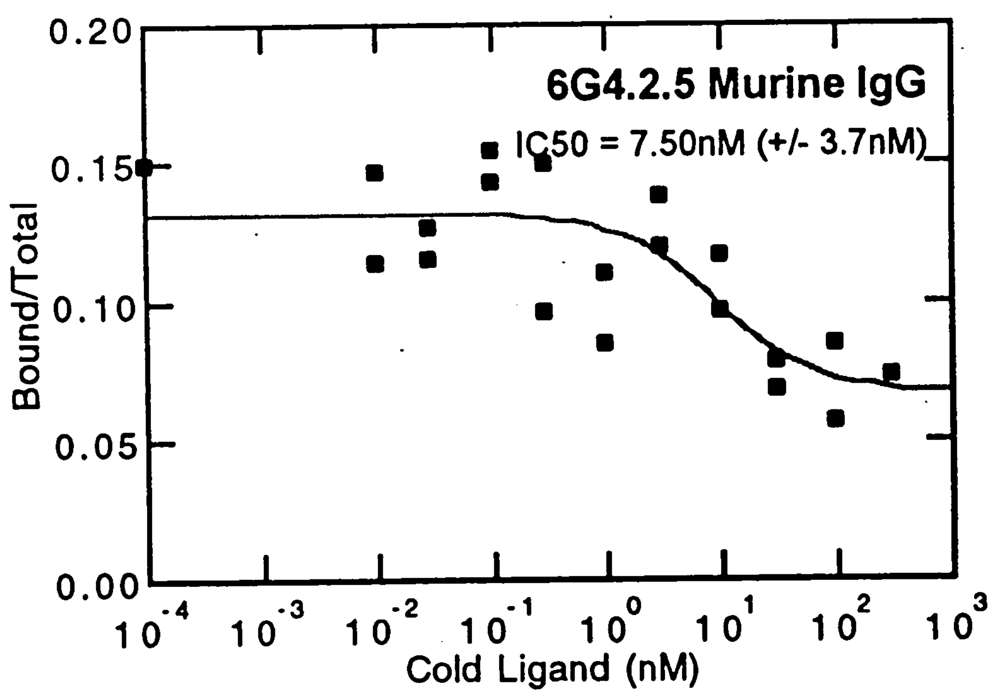


FIG. 49B

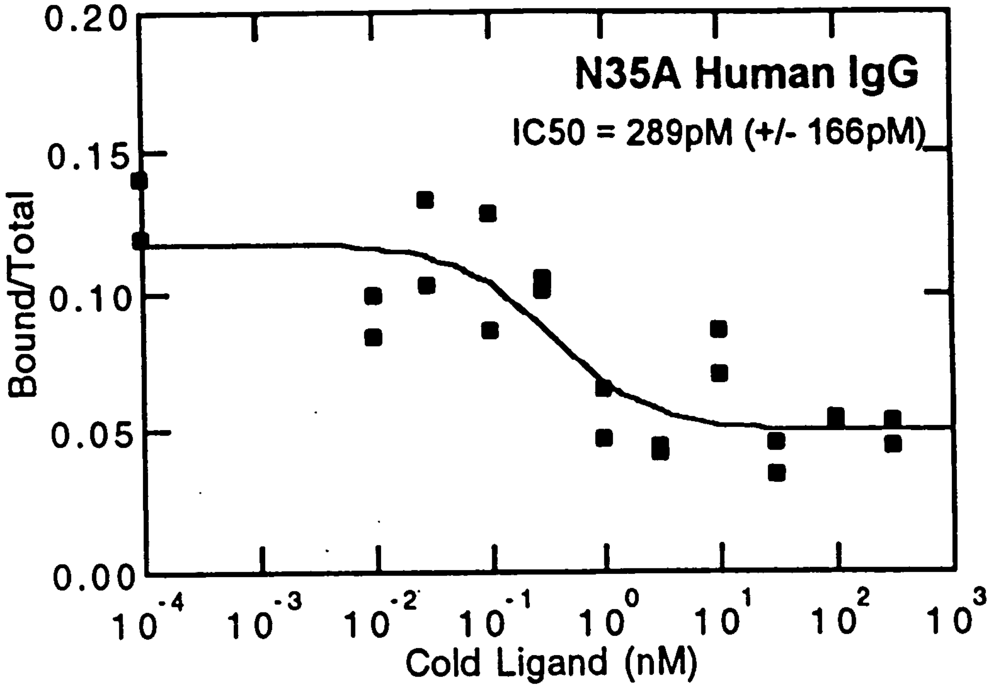


FIG. 49C

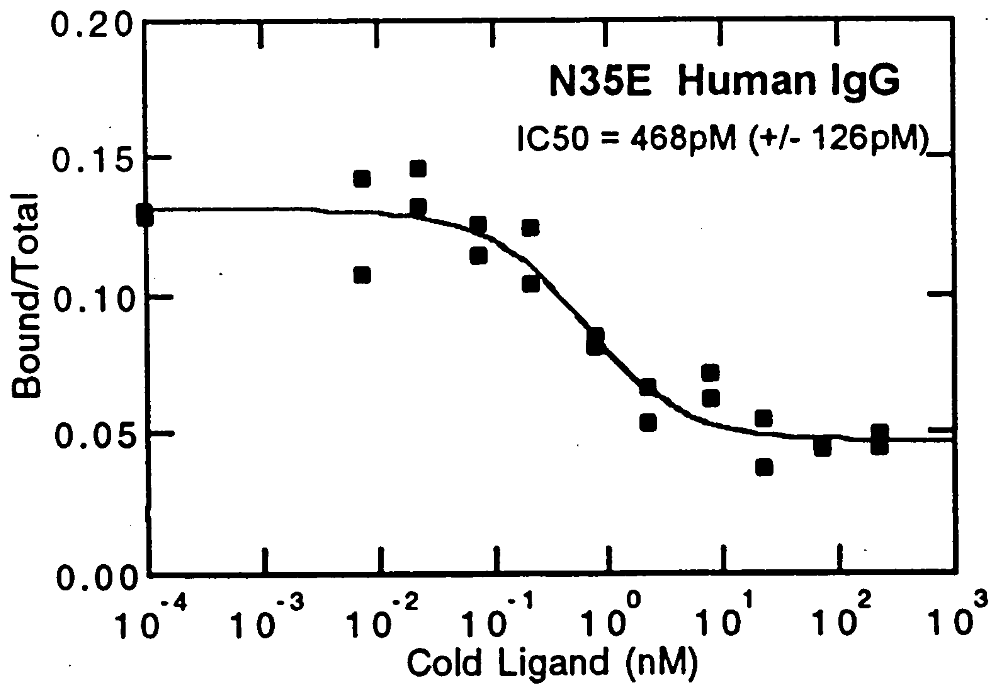
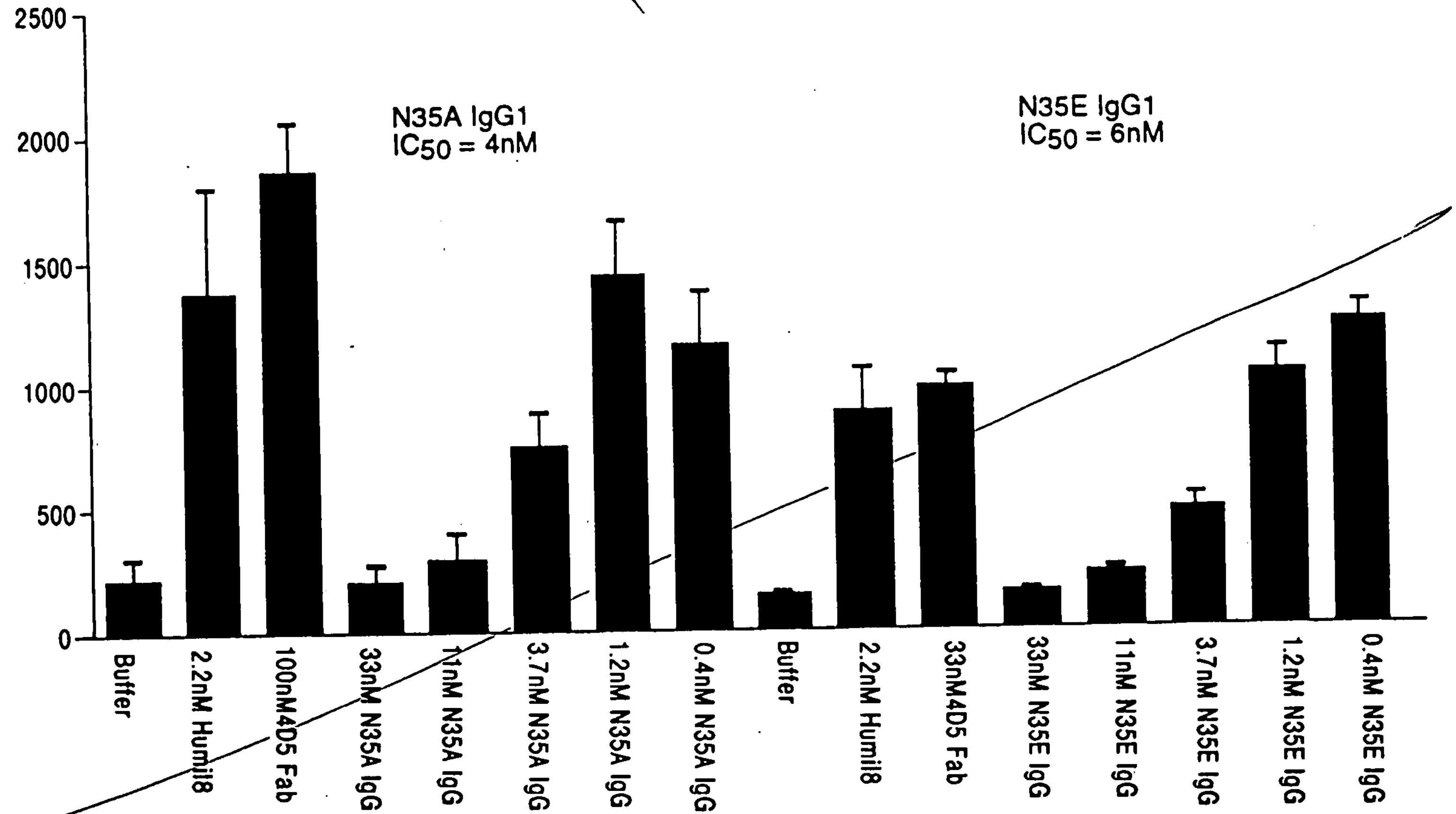


FIG. 49D



2500
2000
1500
1000
500
0
N35A IgG1
IC_{50} = 4nM
N35E IgG1
IC_{50} = 6nM
Buffer
2.2nM Humil8
100nM4D5 Fab
33nM N35A IgG
11nM N35A IgG
3.7nM N35A IgG
1.2nM N35A IgG
0.4nM N35A IgG
Buffer
2.2nM Humil8
33nM4D5 Fab
33nM N35E IgG
11nM N35E IgG
3.7nM N35E IgG
1.2nM N35E IgG
0.4nM N35E IgG

FIG. 50A

00T2T0"10E09T60

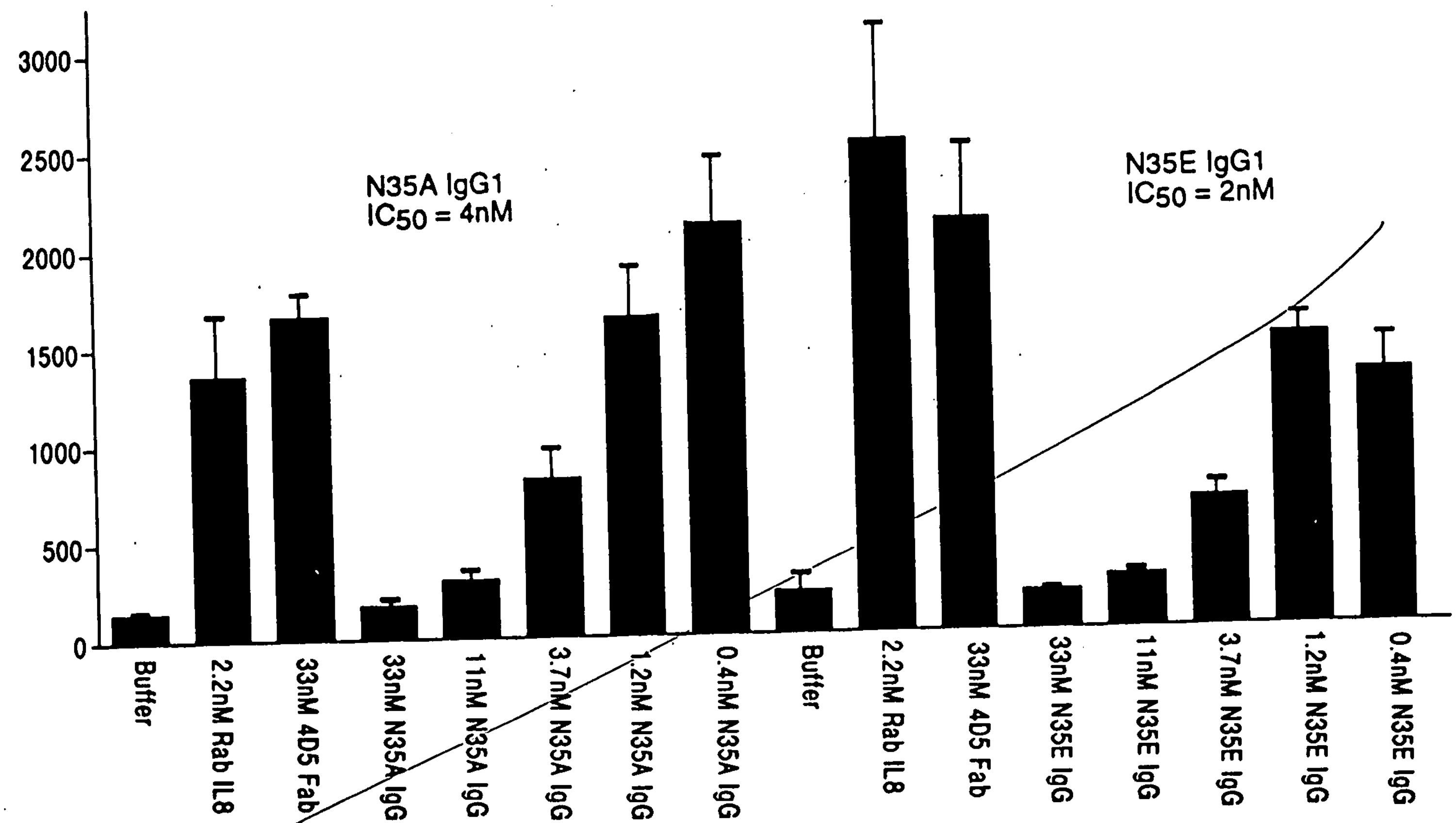
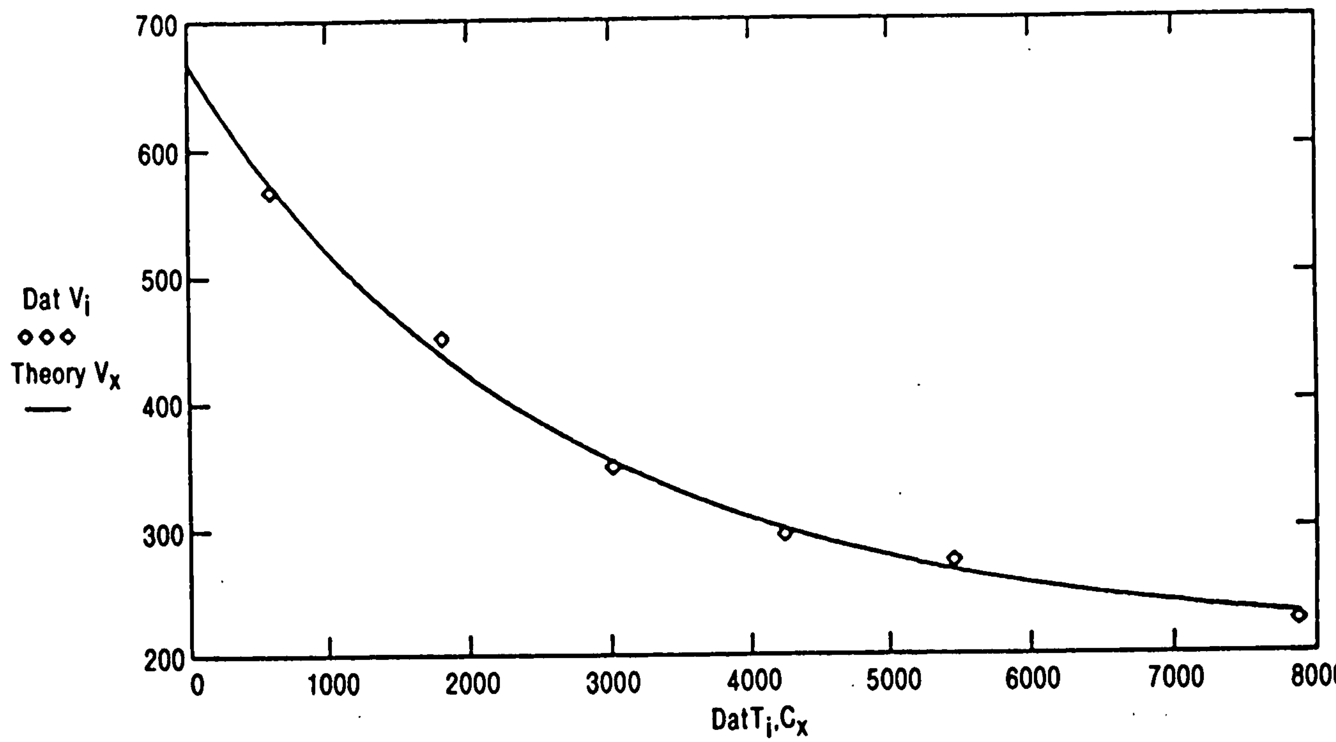


FIG. 50B



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.IgG1

SAMPLE	ka	kd	Kd
Murine 6G4.2.5 IgG2a	8.3×10^5	2.9×10^{-4}	350pM
6G4V11N35A-IgG1	8.7×10^5	7.7×10^{-5}	88pM
6G4V11N35E-IgG1	3.0×10^6	1.4×10^{-4}	49pM

FIG. 51

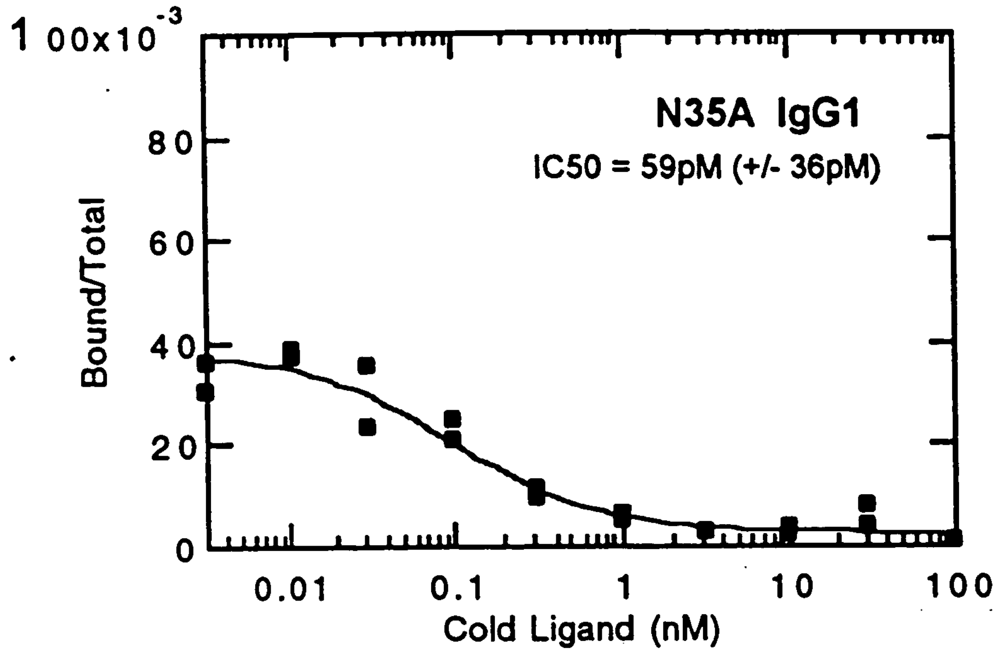


FIG. 52A

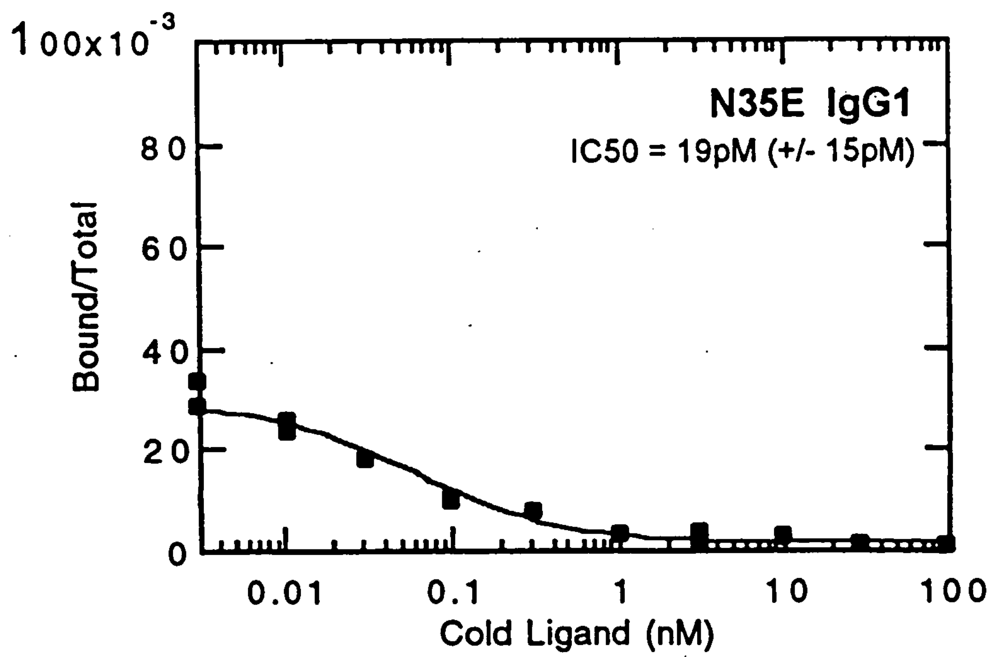


FIG. 52B

```
781  AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
     TTTTCCCATA GATCTCCAAC TCCACTAAAA TACTTTTTCT TATAGCGTAA AGAAGAACGT
 -1                                   M  K  K  N  I  A  F  L  L  A

841  TCTATGTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG AGGTTCAGCT AGTGCAGTCT
     AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
-11  S  M  F  V  F  S  I  A  T  N  A  Y  A  E  V  Q  L  V  Q  S

901  GGCGGTGGCC TGGTGCAGCC AGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
     CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
  8  G  G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A  A  S  G  Y

961  TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
     AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTCCC GGACCTTACC
 28  S  F  S  S  H  Y  M  H  W  V  R  Q  A  P  G  K  G  L  E  W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
     CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
 48  V  G  Y  I  D  P  S  N  G  E  T  T  Y  N  Q  K  F  K  G  R

1081 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
     AAGTGAAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
 68  F  T  L  S  R  D  N  S  K  N  T  A  Y  L  Q  M  N  S  L  R

1141 GCTGAGGACA CTGCCGTCTA TTACTGTGCA AGAGGGGATT ATCGCTACAA TGGTGACTGG
     CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
 88  A  E  D  T  A  V  Y  Y  C  A  R  G  D  Y  R  Y  N  G  D  W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
     AAGAAGCTGC AGACCCCAGT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
108  F  F  D  V  W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
128  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

1321 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
148  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A

1381 CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
     GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
168  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
     TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC
188  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
     TTAGTGTTCG GGTCGTTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
208  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K

1561 ACTCACACAT GCCCGCCGTGA
     TGAGTGTGTA CGGGCGGCACT
228  T  H  T  C  P  P  O
```

FIG. 53

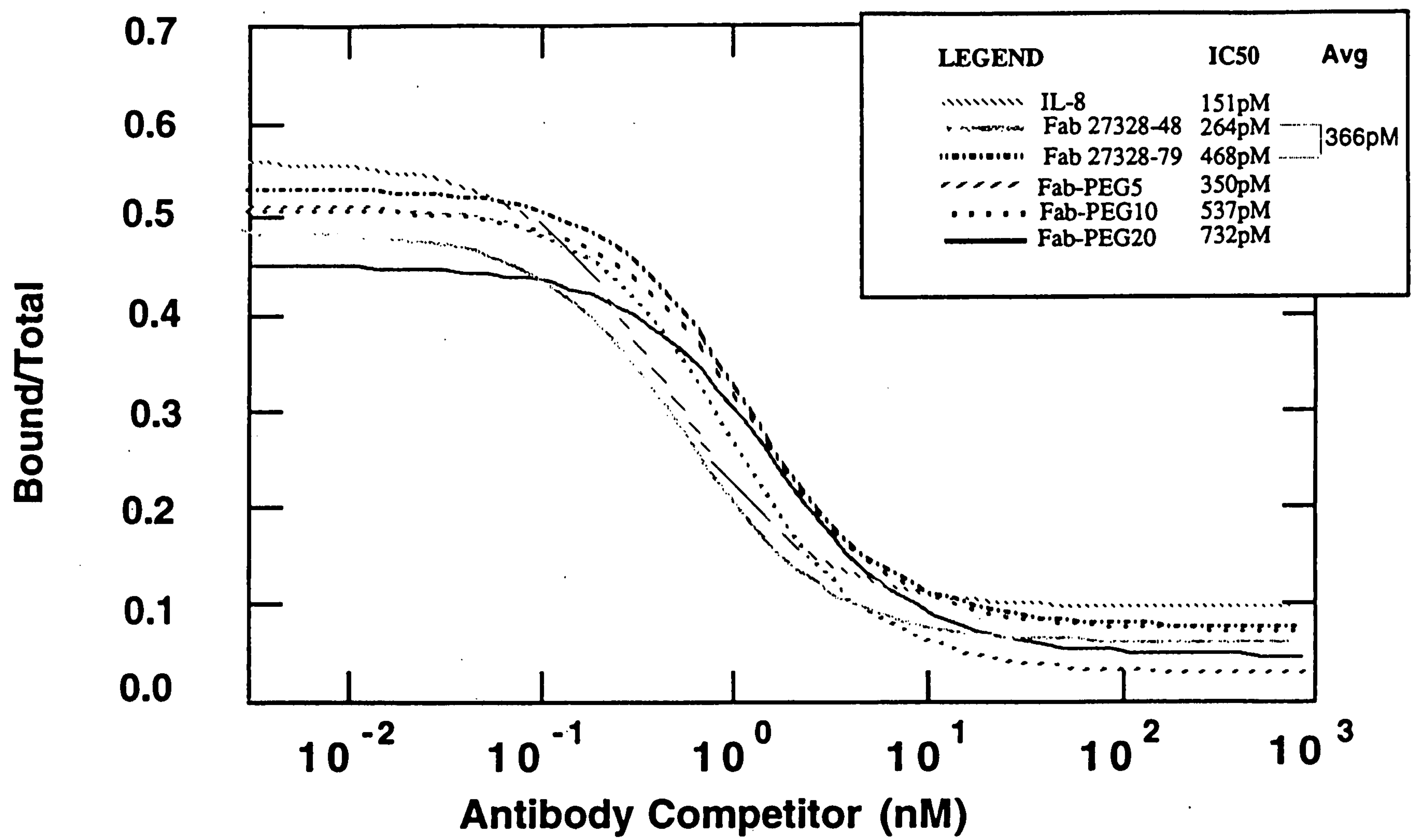
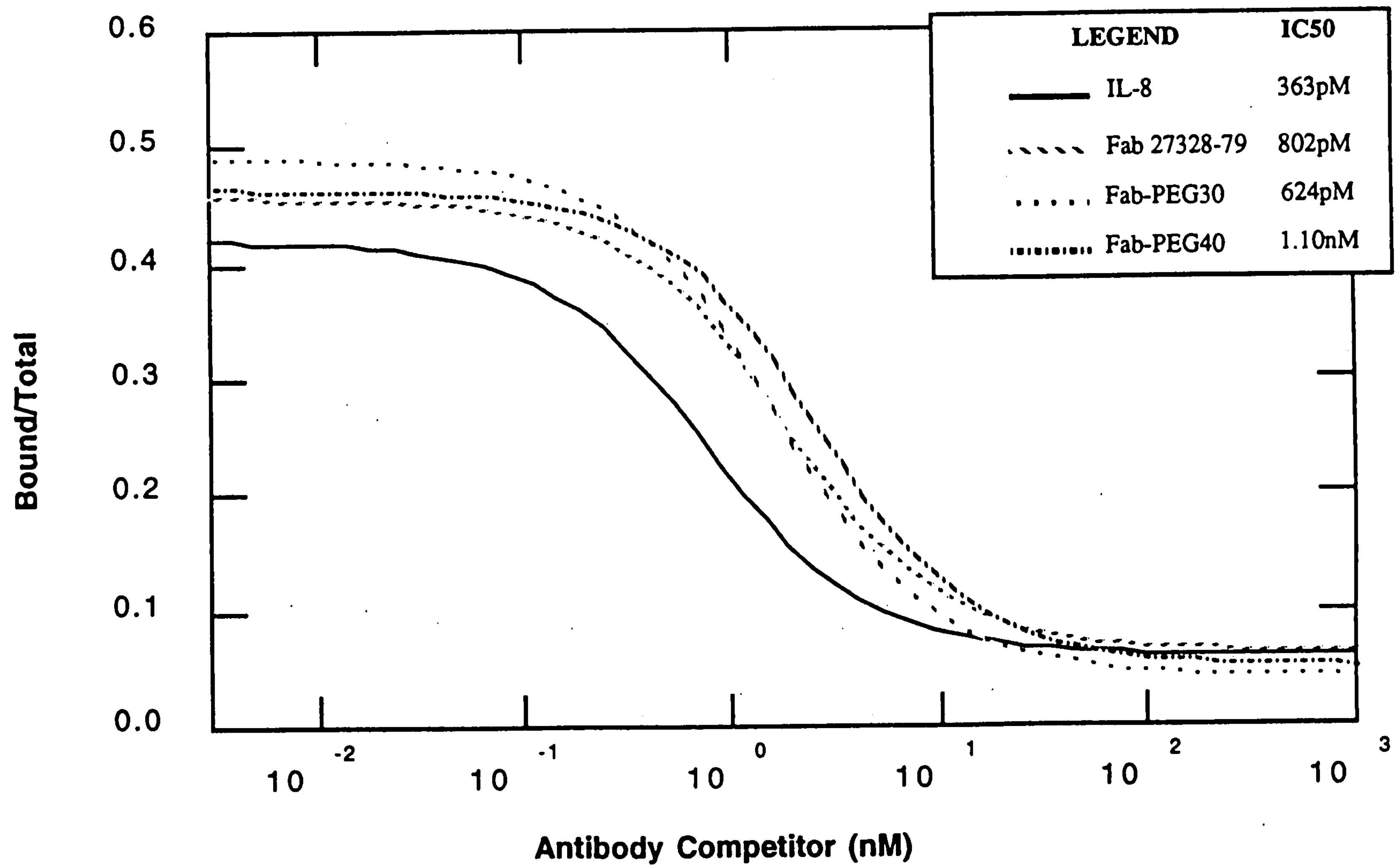
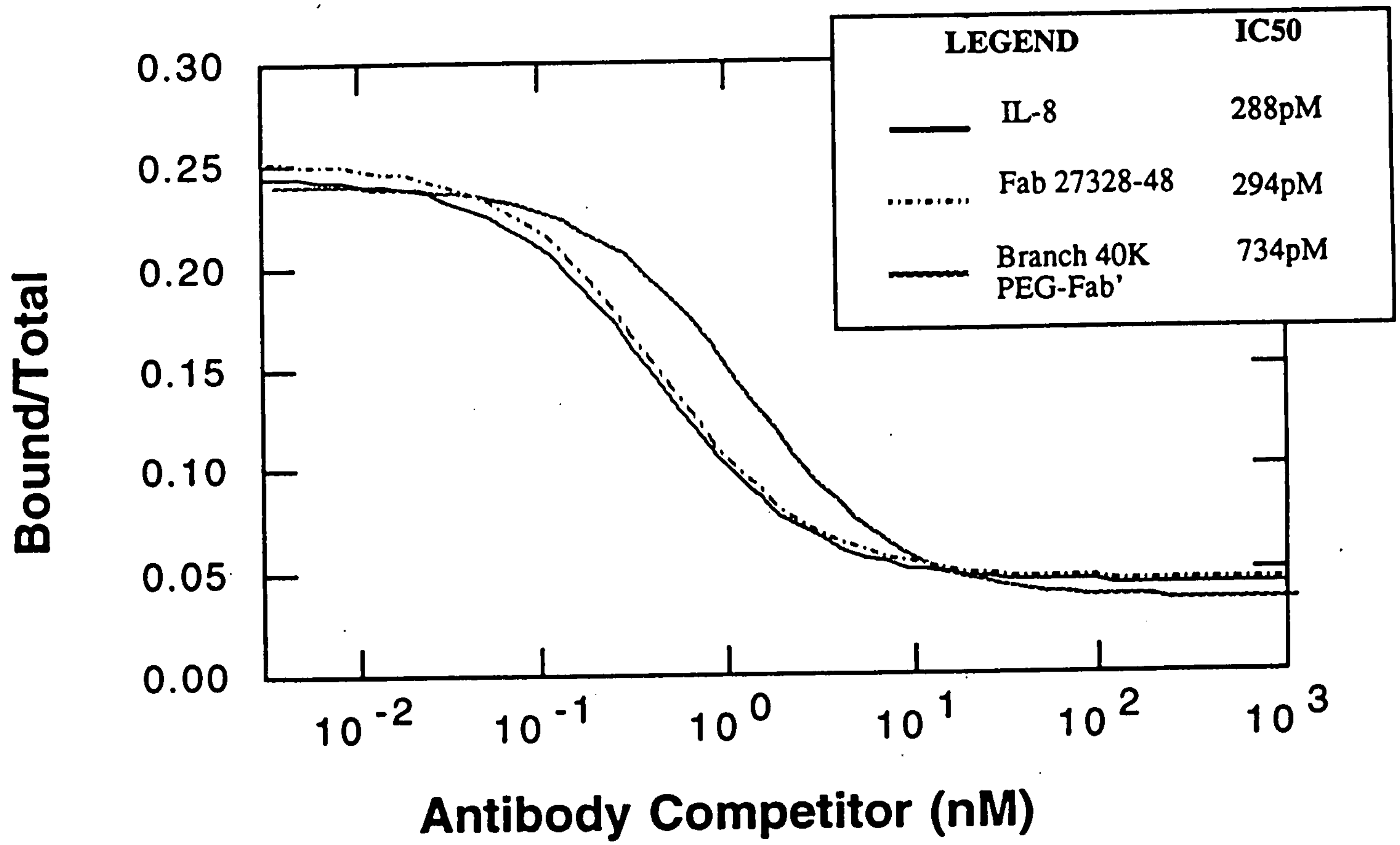

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Bound/Total
LEGEND
IC50
Avg
IL-8
151pM
Fab 27328-48
264pM
366pM
Fab 27328-79
468pM
Fab-PEG5
350pM
Fab-PEG10
537pM
Fab-PEG20
732pM
10 -2
10 -1
10 0
10 1
10 2
10 3
Antibody Competitor (nM)

FIG. 54A



LEGEND
IC50
IL-8
363pM
Fab 27328-79
802pM
Fab-PEG30
624pM
Fab-PEG40
1.10nM
Bound/Total
0.0
0.1
0.2
0.3
0.4
0.5
0.6
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Antibody Competitor (nM)

FIG. 54B



LEGEND
IC50
IL-8
288pM
Fab 27328-48
294pM
Branch 40K PEG-Fab'
734pM
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Bound/Total
10 -2
10 -1
10 0
10 1
10 2
10 3
Antibody Competitor (nM)

FIG. 54C

00T2T0"10C08100

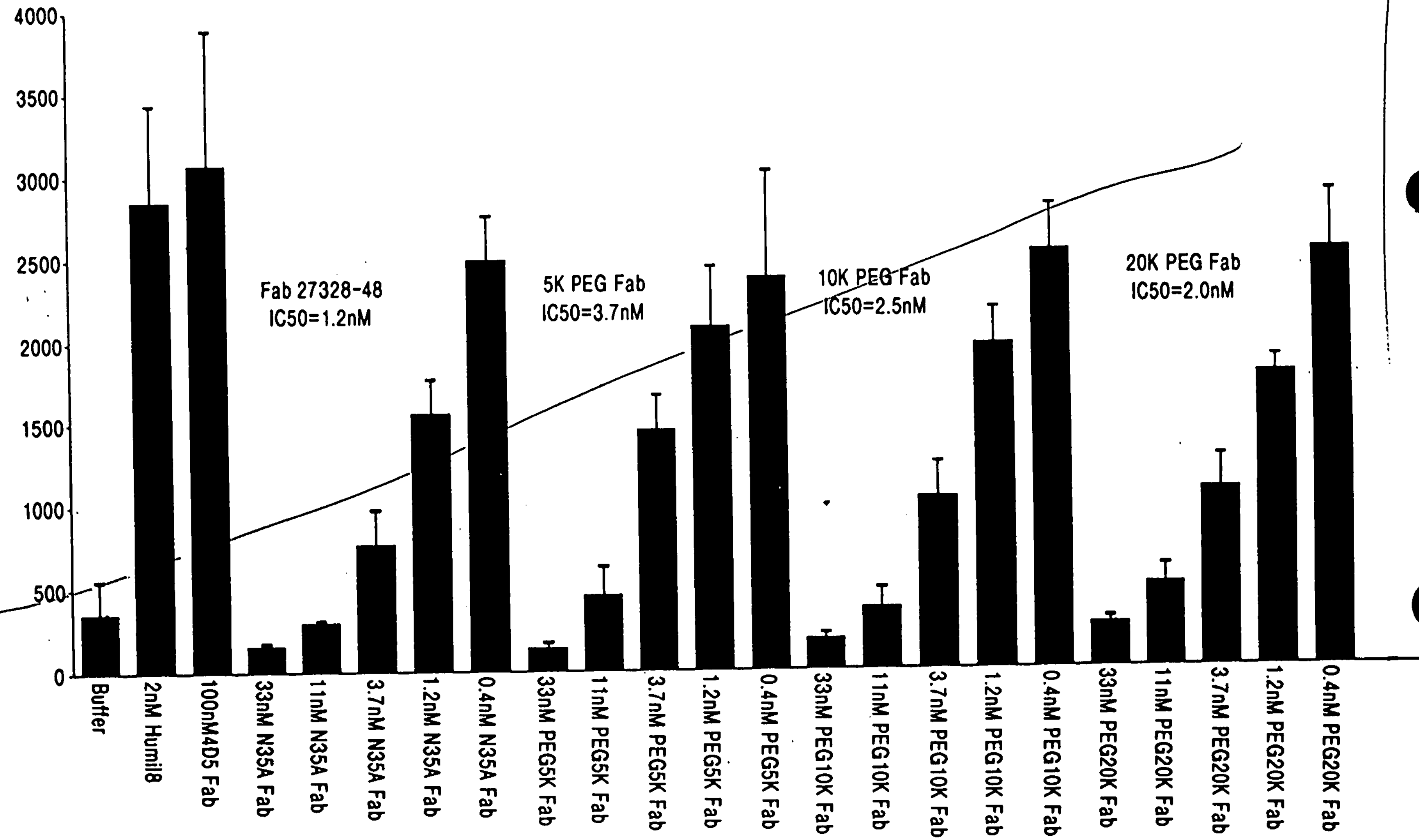


FIG. 55A

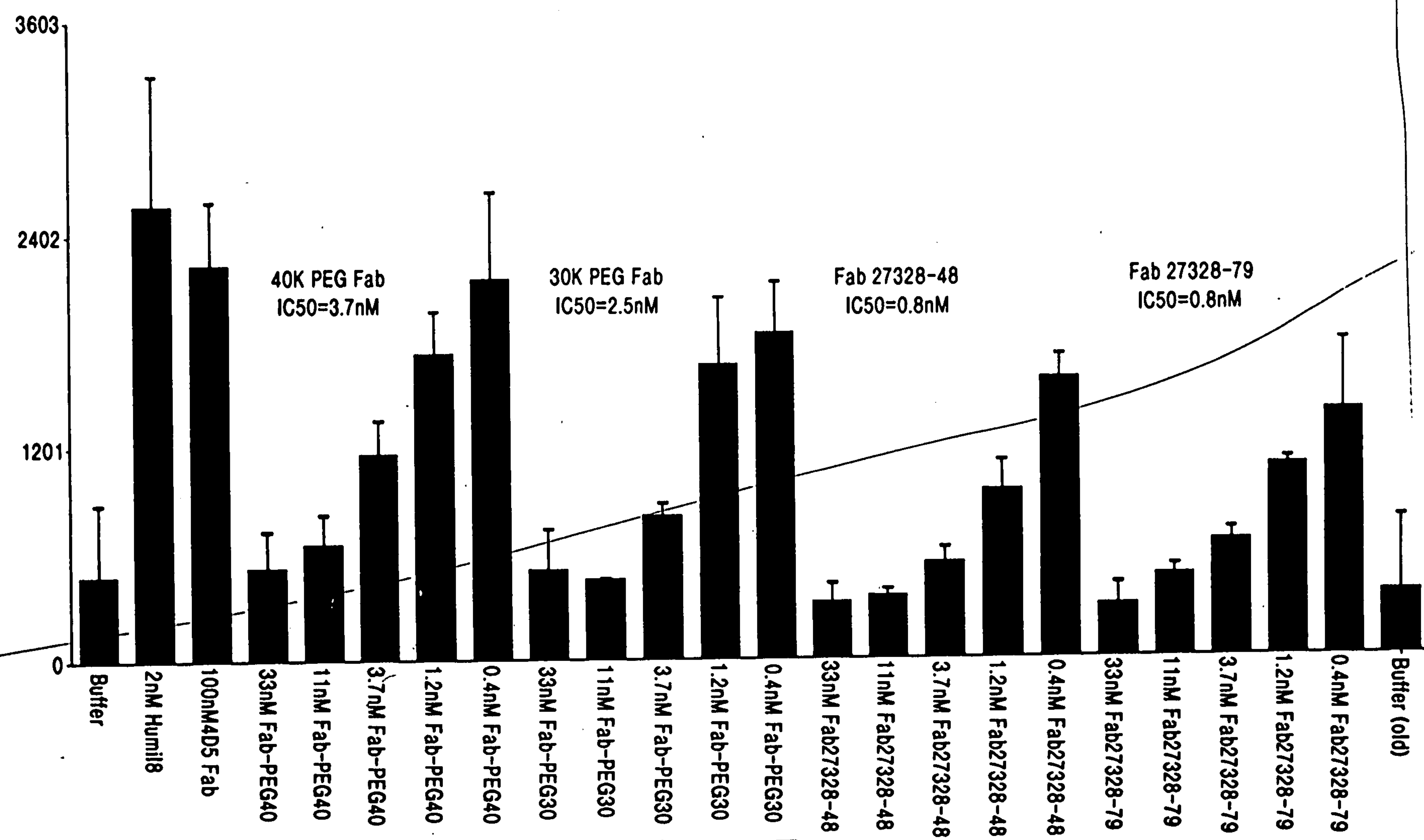

3603
2402
1201
0
40K PEG Fab
IC50=3.7nM
30K PEG Fab
IC50=2.5nM
Fab 27328-48
IC50=0.8nM
Fab 27328-79
IC50=0.8nM
Buffer
2nM Humil8
100nM4D5 Fab
33nM Fab-PEG40
11nM Fab-PEG40
3.7nM Fab-PEG40
1.2nM Fab-PEG40
0.4nM Fab-PEG40
33nM Fab-PEG30
11nM Fab-PEG30
3.7nM Fab-PEG30
1.2nM Fab-PEG30
0.4nM Fab-PEG30
33nM Fab27328-48
11nM Fab27328-48
3.7nM Fab27328-48
1.2nM Fab27328-48
0.4nM Fab27328-48
33nM Fab27328-79
11nM Fab27328-79
3.7nM Fab27328-79
1.2nM Fab27328-79
0.4nM Fab27328-79
Buffer (old)

FIG. 55B

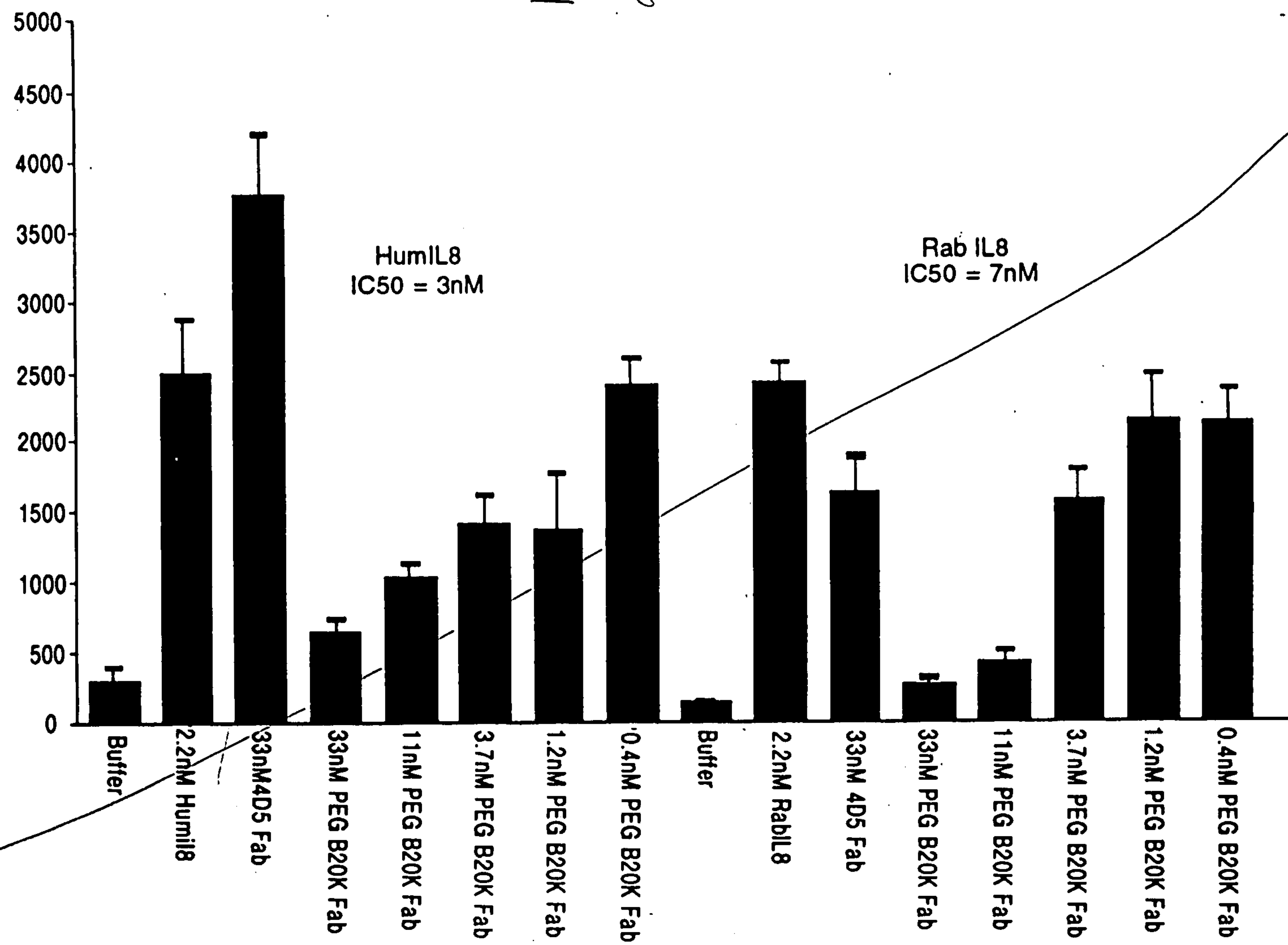
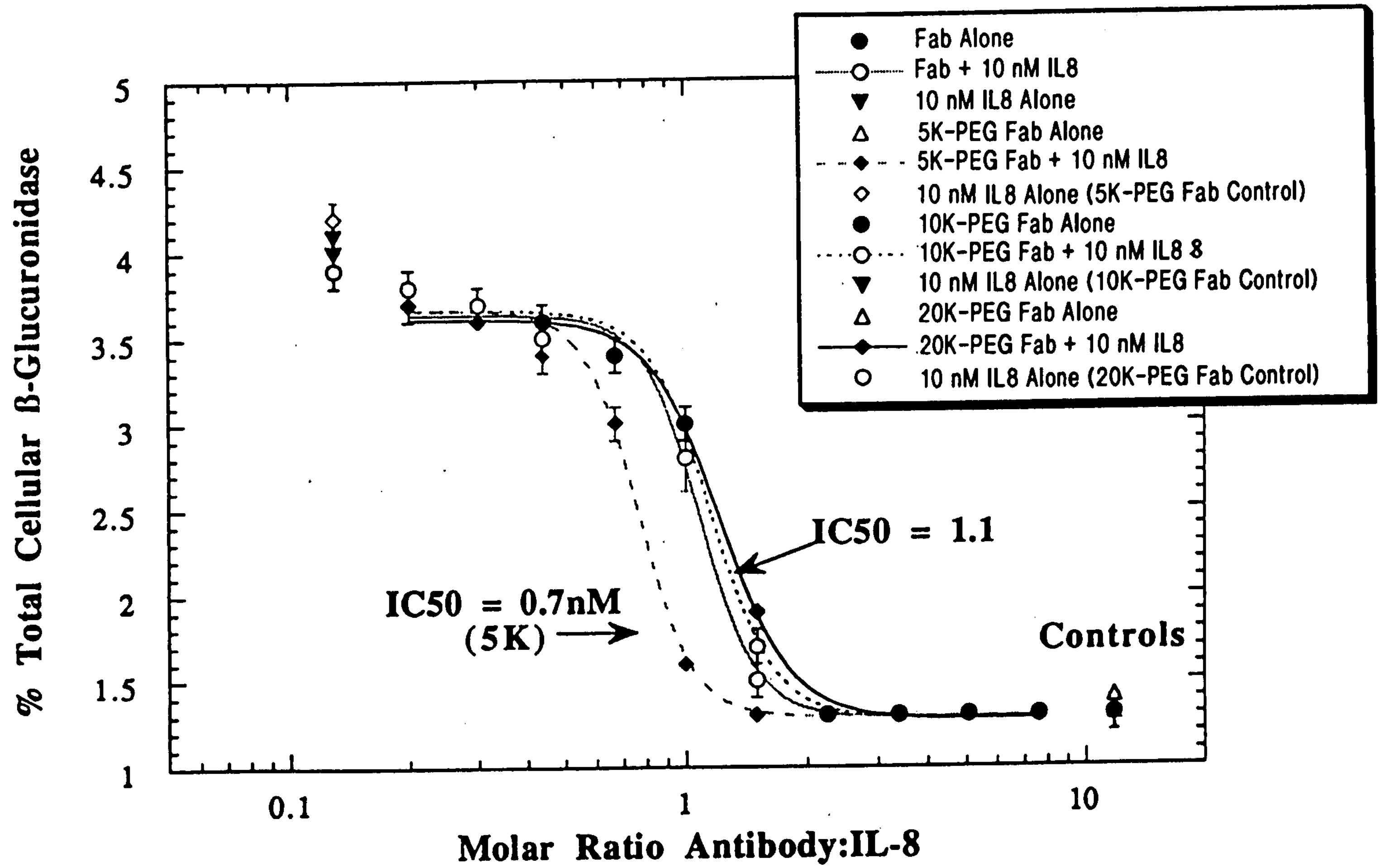
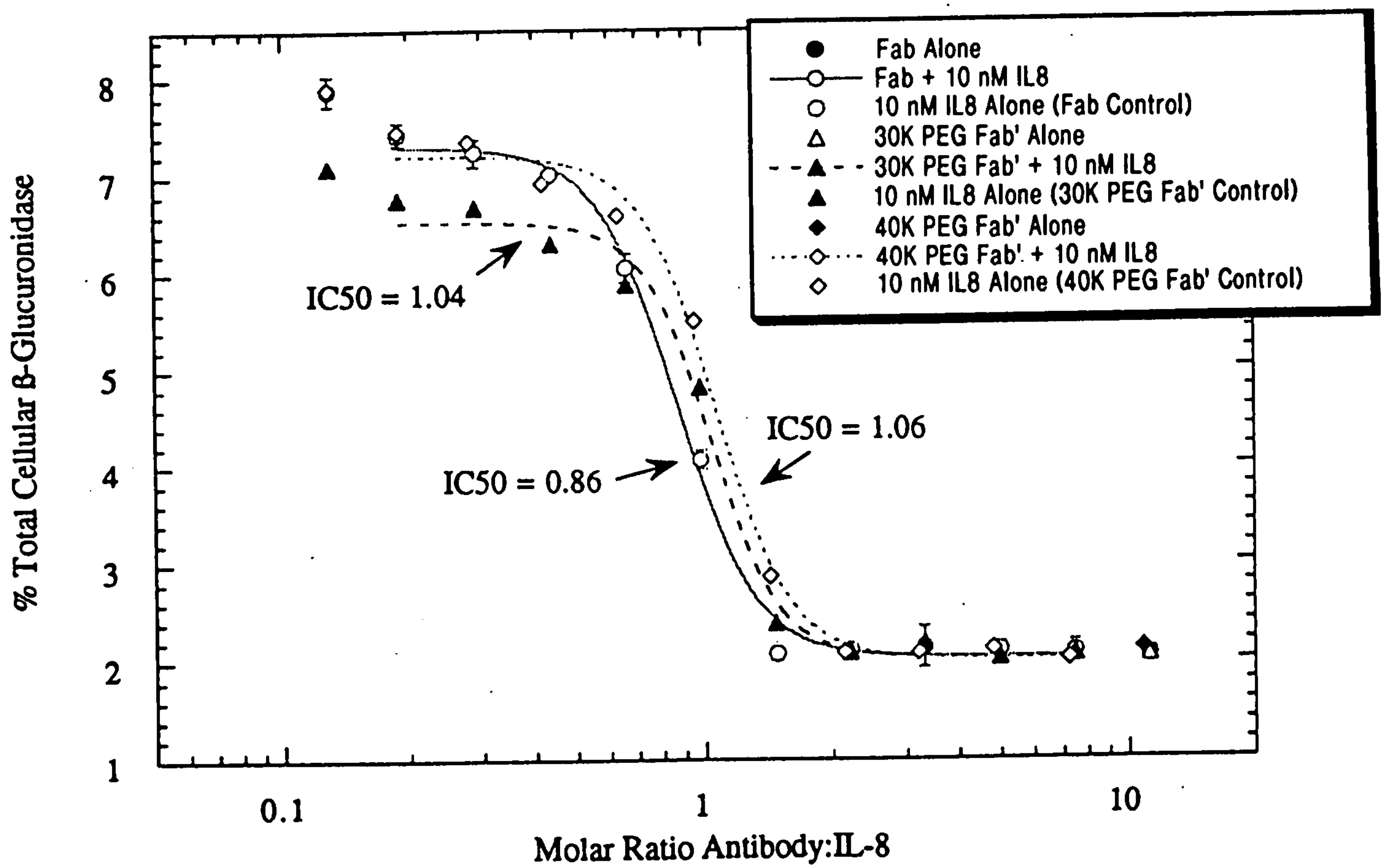

5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
HumIL8
IC50 = 3nM
Rab IL8
IC50 = 7nM
Buffer
2.2nM Humil8
33nM4D5 Fab
33nM PEG B20K Fab
11nM PEG B20K Fab
3.7nM PEG B20K Fab
1.2nM PEG B20K Fab
0.4nM PEG B20K Fab
Buffer
2.2nM RabIL8
33nM 4D5 Fab
33nM PEG B20K Fab
11nM PEG B20K Fab
3.7nM PEG B20K Fab
1.2nM PEG B20K Fab
0.4nM PEG B20K Fab

FIG. 55C



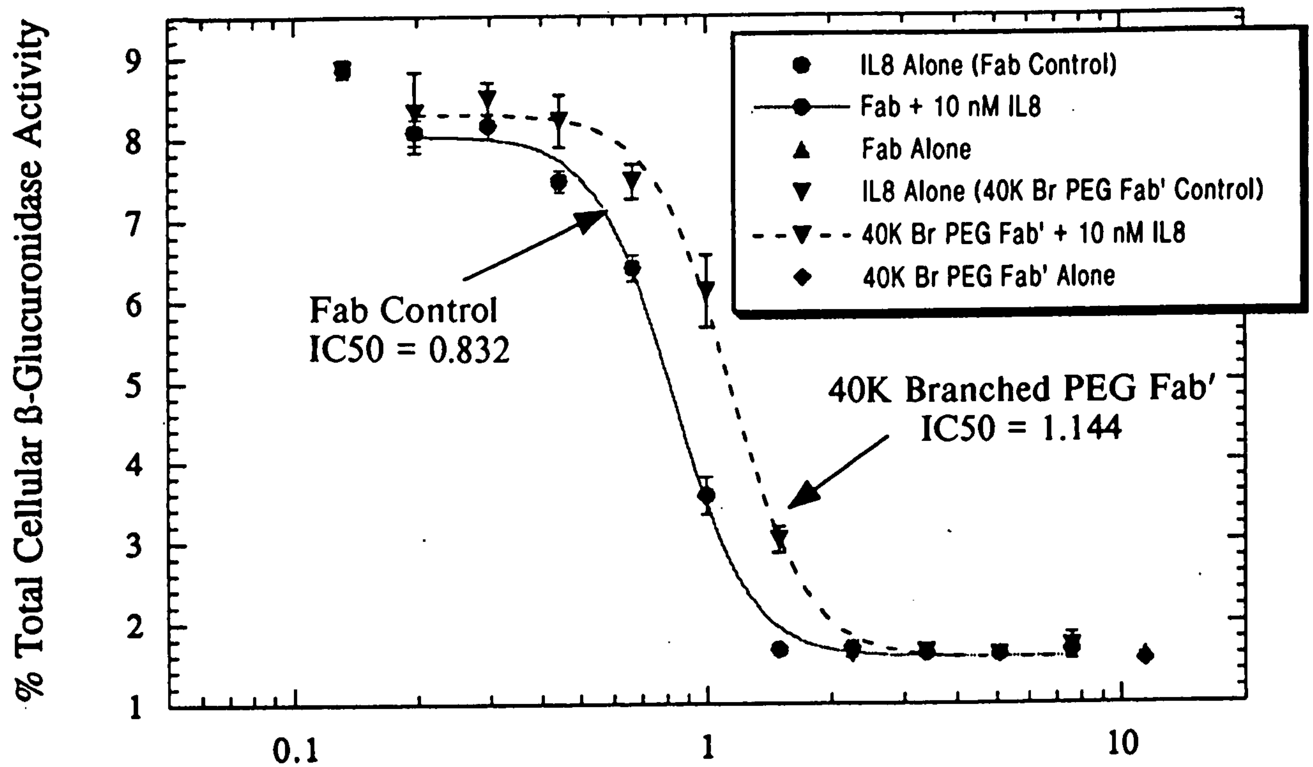
Fab Alone
Fab + 10 nM IL8
10 nM IL8 Alone
5K-PEG Fab Alone
5K-PEG Fab + 10 nM IL8
10 nM IL8 Alone (5K-PEG Fab Control)
10K-PEG Fab Alone
10K-PEG Fab + 10 nM IL8 8
10 nM IL8 Alone (10K-PEG Fab Control)
20K-PEG Fab Alone
20K-PEG Fab + 10 nM IL8
10 nM IL8 Alone (20K-PEG Fab Control)
% Total Cellular ß-Glucuronidase
5
4.5
4
3.5
3
2.5
2
1.5
1
0.1
1
10
IC50 = 1.1
IC50 = 0.7nM
(5K)
Controls
Molar Ratio Antibody:IL-8

FIG. 56A



Fab Alone
Fab + 10 nM IL8
10 nM IL8 Alone (Fab Control)
30K PEG Fab' Alone
30K PEG Fab' + 10 nM IL8
10 nM IL8 Alone (30K PEG Fab' Control)
40K PEG Fab' Alone
40K PEG Fab' + 10 nM IL8
10 nM IL8 Alone (40K PEG Fab' Control)
IC50 = 1.04
IC50 = 0.86
IC50 = 1.06
% Total Cellular ß-Glucuronidase
Molar Ratio Antibody:IL-8
8
7
6
5
4
3
2
1
0.1
1
10

FIG. 56B



IL8 Alone (Fab Control)
Fab + 10 nM IL8
Fab Alone
IL8 Alone (40K Br PEG Fab' Control)
40K Br PEG Fab' + 10 nM IL8
40K Br PEG Fab' Alone
Fab Control
IC50 = 0.832
40K Branched PEG Fab'
IC50 = 1.144
% Total Cellular ß-Glucuronidase Activity
Molar Ratio Antibody:IL8
0.1
1
10
1
2
3
4
5
6
7
8
9

FIG. 56C

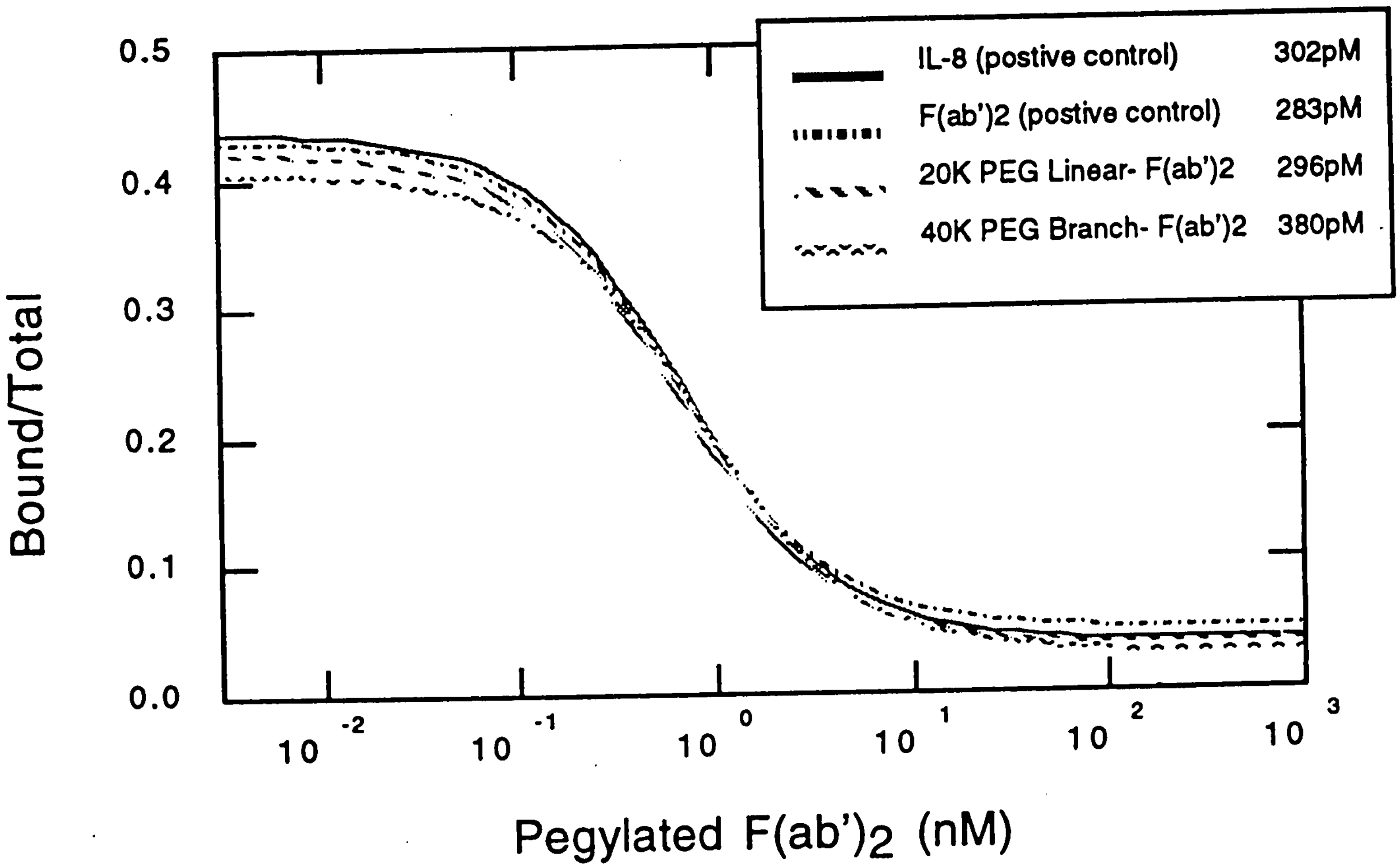
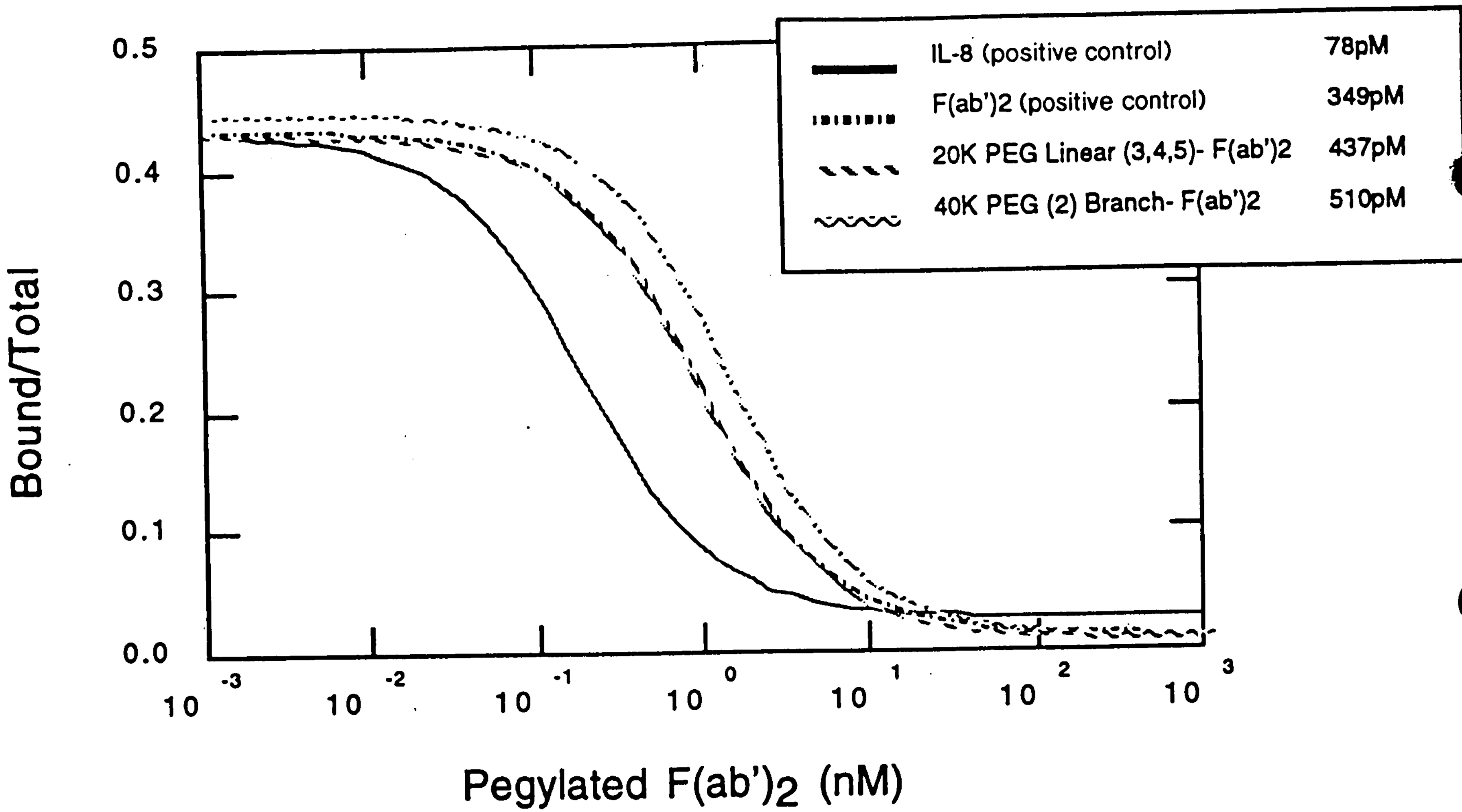
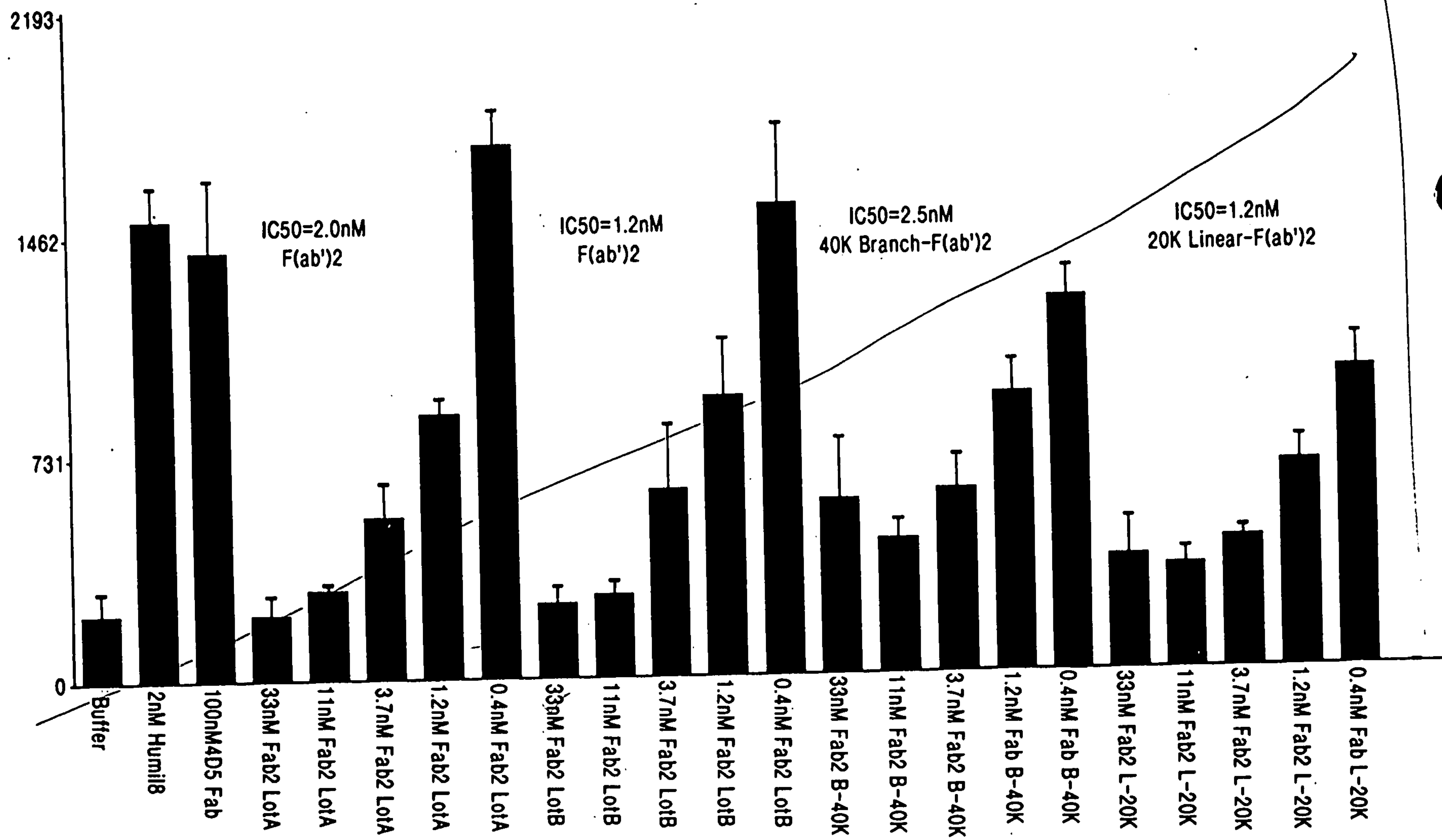

0.5
0.4
0.3
0.2
0.1
0.0
Bound/Total
IL-8 (postive control) 302pM
F(ab')2 (postive control) 283pM
20K PEG Linear- F(ab')2 296pM
40K PEG Branch- F(ab')2 380pM
10 -2
10 -1
10 0
10 1
10 2
10 3
Pegylated F(ab')2 (nM)

FIG. 57A



IL-8 (positive control) 78pM
F(ab')2 (positive control) 349pM
20K PEG Linear (3,4,5)- F(ab')2 437pM
40K PEG (2) Branch- F(ab')2 510pM
Bound/Total
0.5
0.4
0.3
0.2
0.1
0.0
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3
Pegylated F(ab')2 (nM)

FIG. 57B



2193
1462
731
0
IC50=2.0nM
F(ab')2
IC50=1.2nM
F(ab')2
IC50=2.5nM
40K Branch-F(ab')2
IC50=1.2nM
20K Linear-F(ab')2
Buffer
2nM Humil8
100nM4D5 Fab
33nM Fab2 LotA
11nM Fab2 LotA
3.7nM Fab2 LotA
1.2nM Fab2 LotA
0.4nM Fab2 LotA
33nM Fab2 LotB
11nM Fab2 LotB
3.7nM Fab2 LotB
1.2nM Fab2 LotB
0.4nM Fab2 LotB
33nM Fab2 B-40K
11nM Fab2 B-40K
3.7nM Fab2 B-40K
1.2nM Fab B-40K
0.4nM Fab B-40K
33nM Fab2 L-20K
11nM Fab2 L-20K
3.7nM Fab2 L-20K
1.2nM Fab2 L-20K
0.4nM Fab L-20K

FIG. 58A

00T2T0"1CCCG1C0

Amb B13

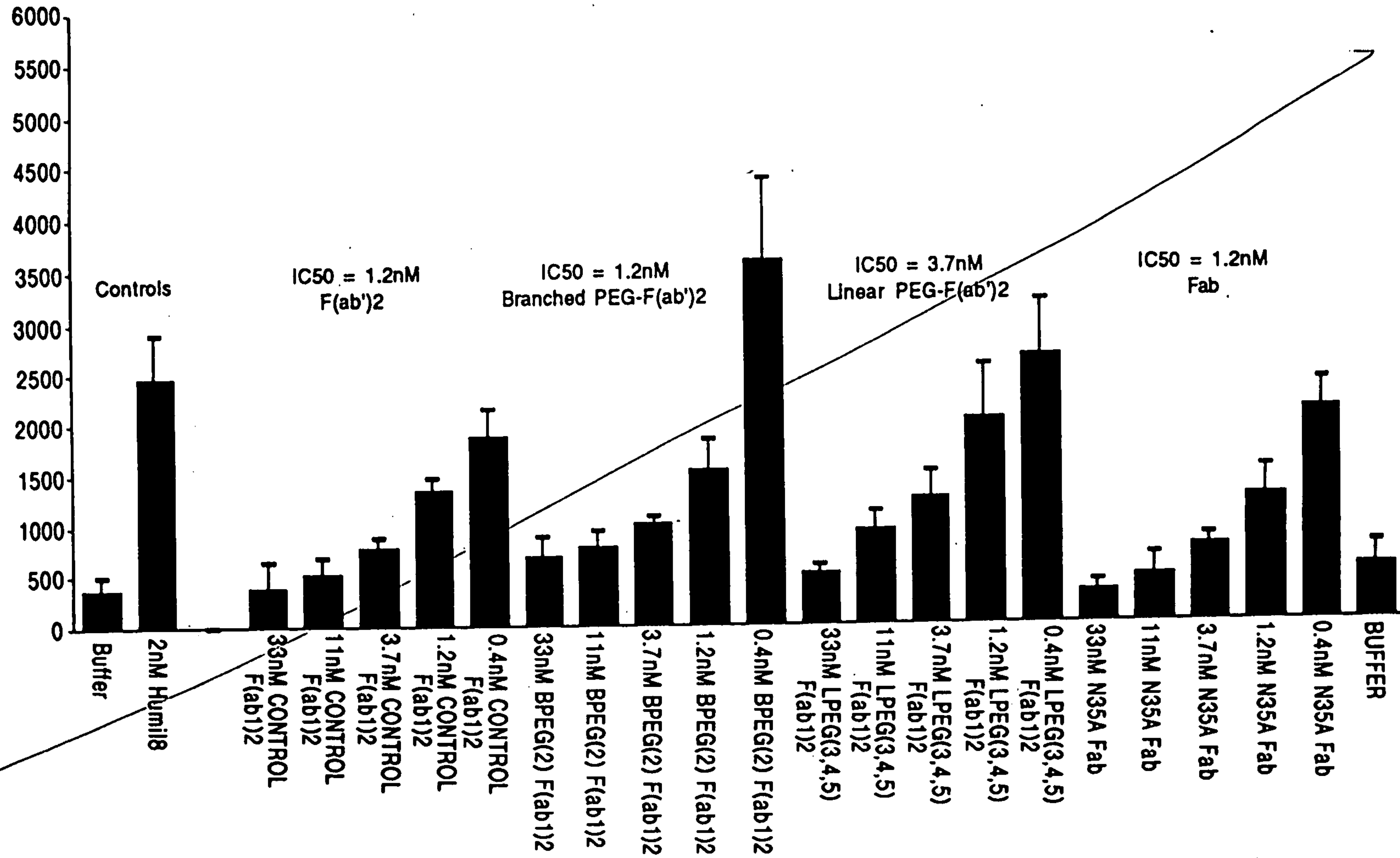


FIG. 58B

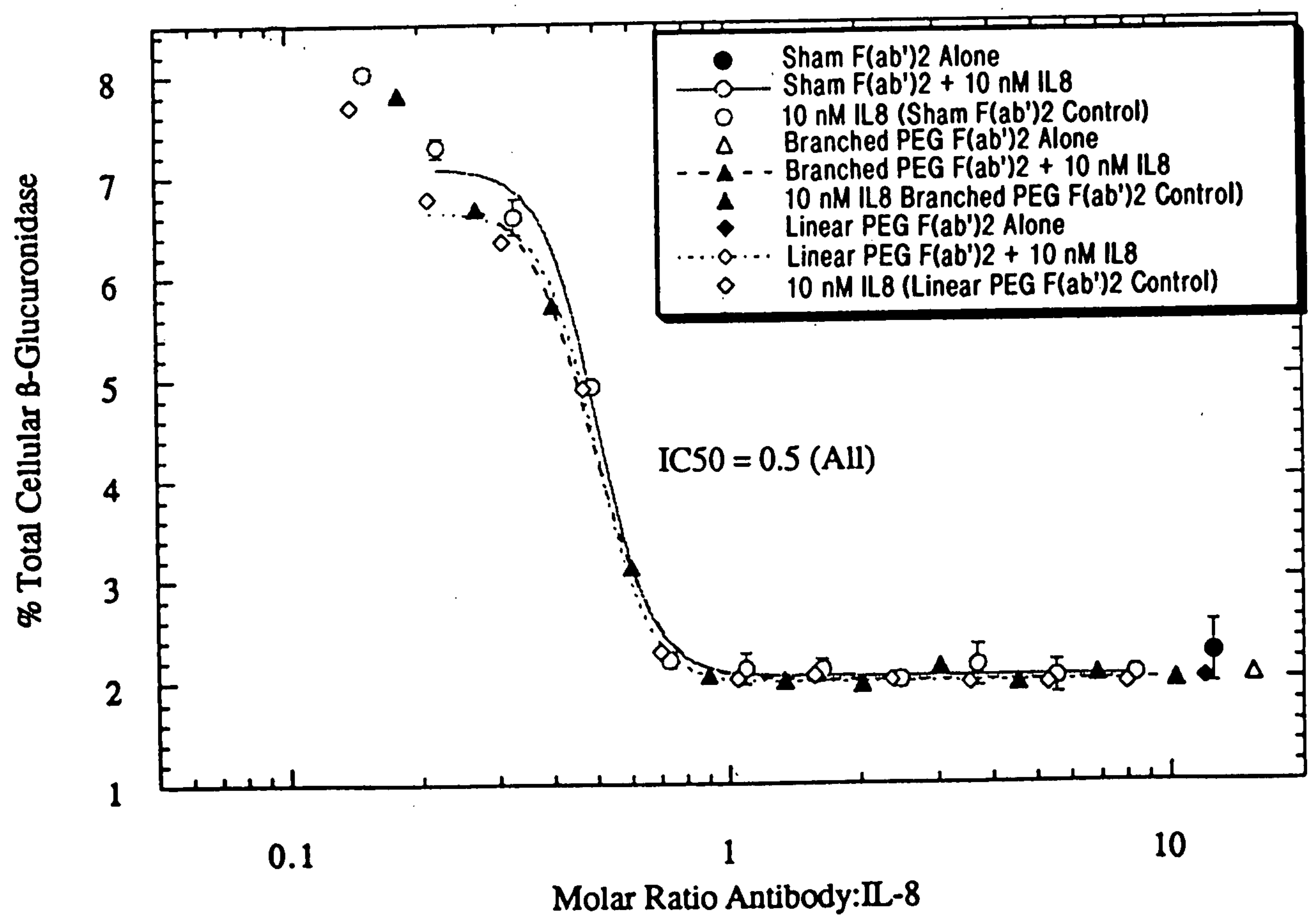

Sham F(ab')2 Alone
Sham F(ab')2 + 10 nM IL8
10 nM IL8 (Sham F(ab')2 Control)
Branched PEG F(ab')2 Alone
Branched PEG F(ab')2 + 10 nM IL8
10 nM IL8 Branched PEG F(ab')2 Control)
Linear PEG F(ab')2 Alone
Linear PEG F(ab')2 + 10 nM IL8
10 nM IL8 (Linear PEG F(ab')2 Control)
IC50 = 0.5 (All)
% Total Cellular ß-Glucuronidase
8
7
6
5
4
3
2
1
0.1
1
10
Molar Ratio Antibody:IL-8

FIG. 59A

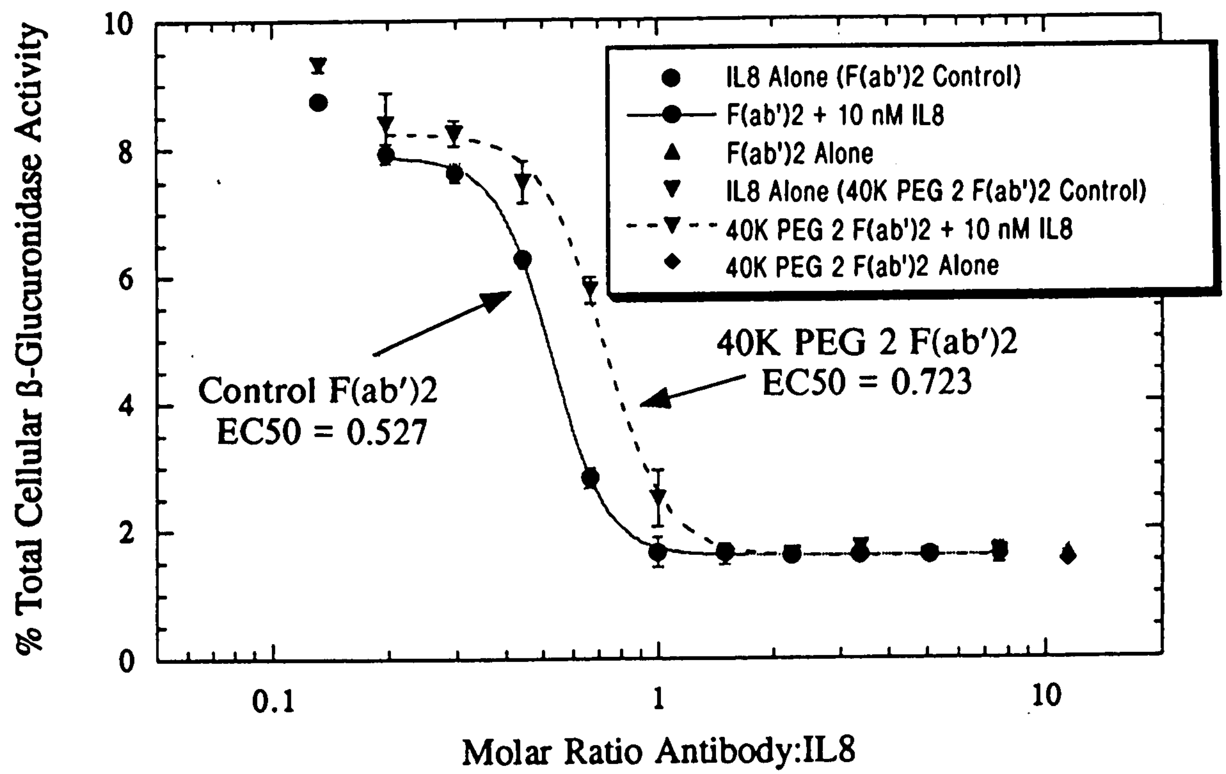
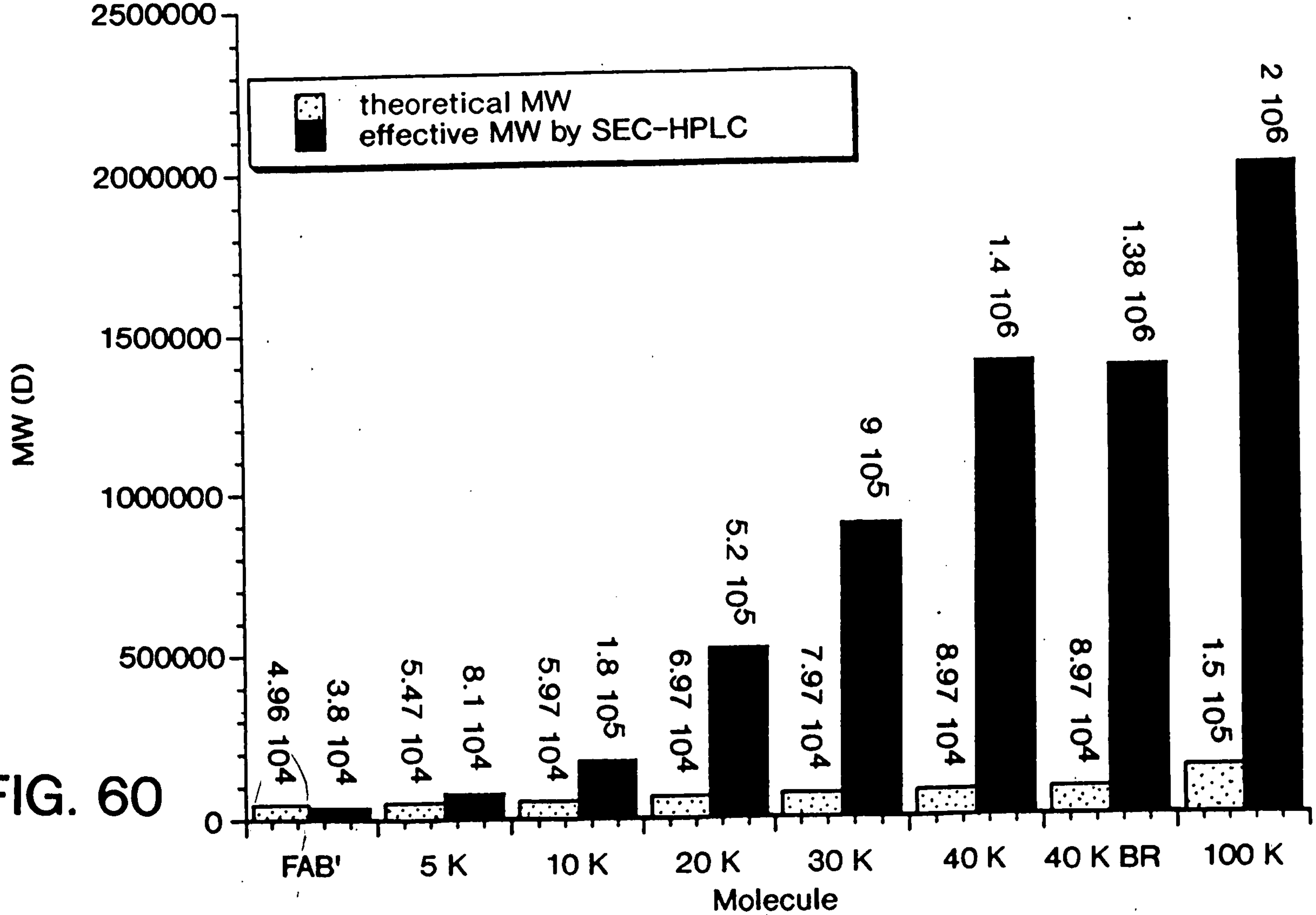

% Total Cellular ß-Glucuronidase Activity
10
8
6
4
2
0
0.1
1
10
Molar Ratio Antibody:IL8
IL8 Alone (F(ab')2 Control)
F(ab')2 + 10 nM IL8
F(ab')2 Alone
IL8 Alone (40K PEG 2 F(ab')2 Control)
40K PEG 2 F(ab')2 + 10 nM IL8
40K PEG 2 F(ab')2 Alone
Control F(ab')2
EC50 = 0.527
40K PEG 2 F(ab')2
EC50 = 0.723

FIG. 59B



theoretical MW
effective MW by SEC-HPLC
MW (D)
2500000
2000000
1500000
1000000
500000
0
4.96 10^4
3.8 10^4
5.47 10^4
8.1 10^4
5.97 10^4
1.8 10^5
6.97 10^4
5.2 10^5
7.97 10^4
9 10^5
8.97 10^4
1.4 10^6
8.97 10^4
1.38 10^6
1.5 10^5
2 10^6
FAB'
5 K
10 K
20 K
30 K
40 K
40 K BR
100 K
Molecule

FIG. 60

Reduced

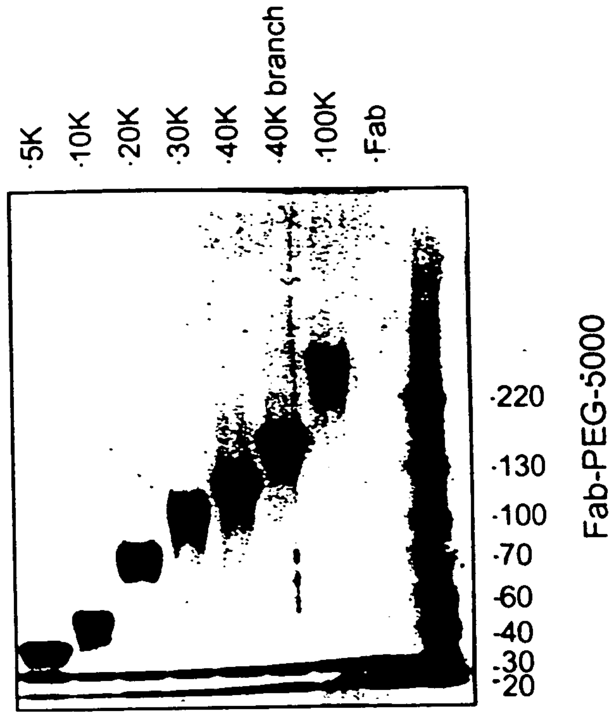


FIG. 61A

Non-Reduced

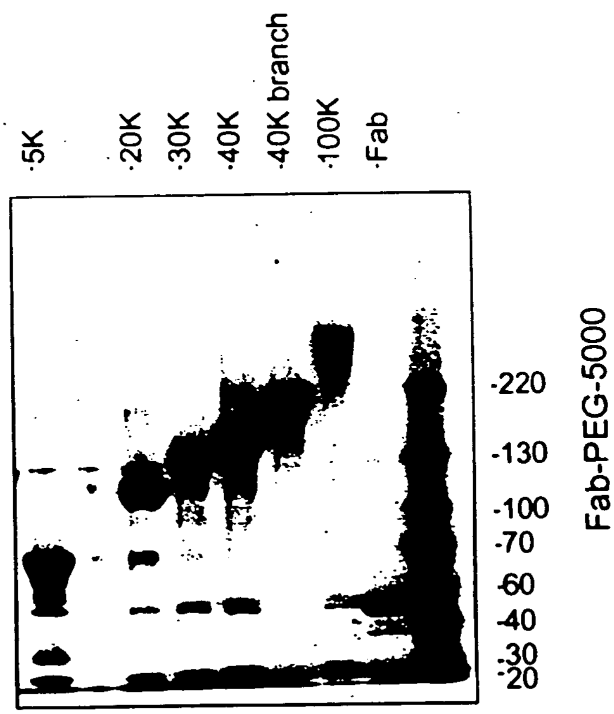
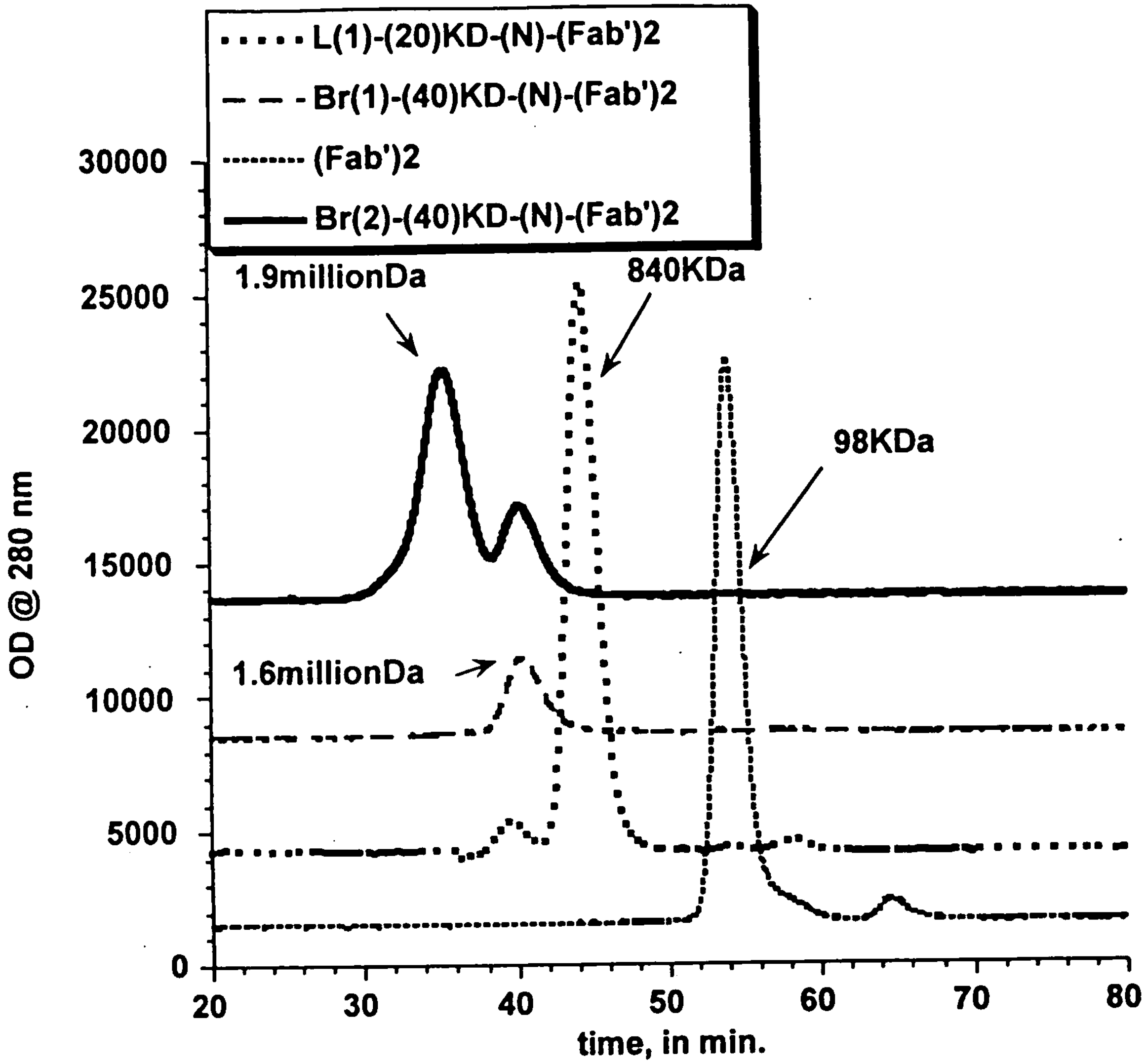


FIG. 61B



L(1)-(20)KD-(N)-(Fab')2
Br(1)-(40)KD-(N)-(Fab')2
(Fab')2
Br(2)-(40)KD-(N)-(Fab')2
30000
25000
20000
15000
10000
5000
0
OD @ 280 nm
1.9millionDa
840KDa
98KDa
1.6millionDa
20
30
40
50
60
70
80
time, in min.

FIG. 62

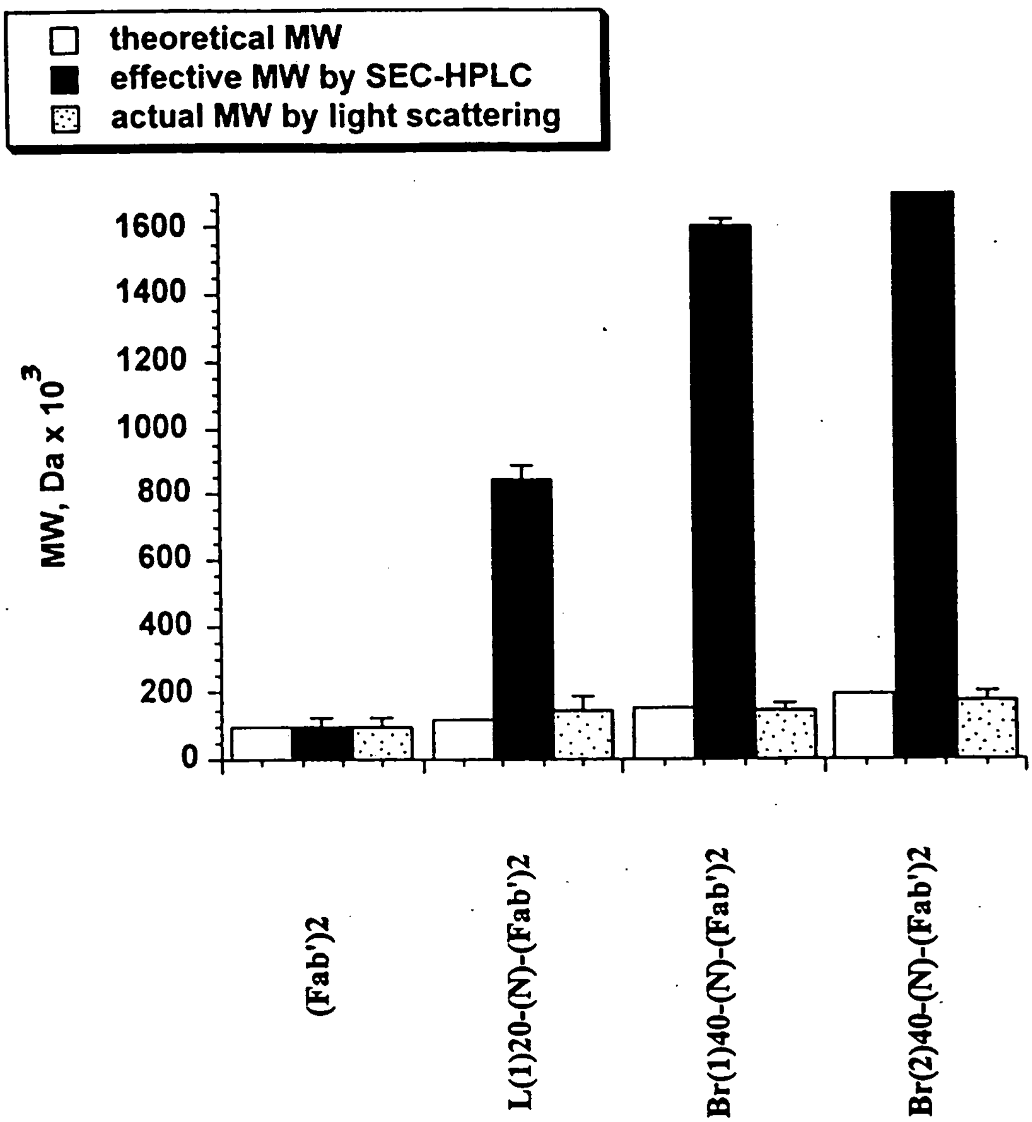
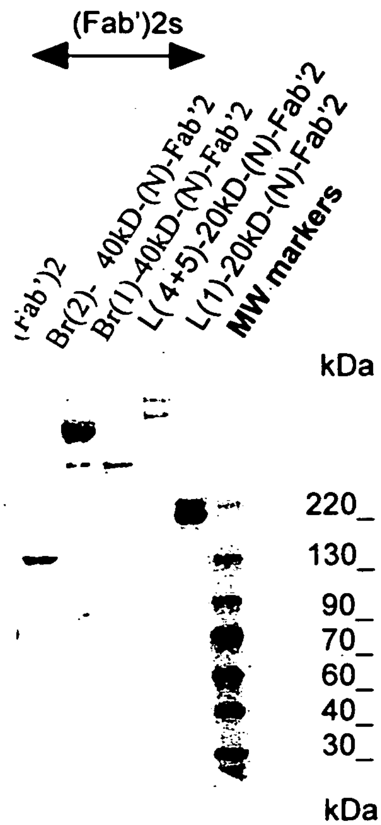

theoretical MW
effective MW by SEC-HPLC
actual MW by light scattering
MW, Da x 10^3
1600
1400
1200
1000
800
600
400
200
0
(Fab')2
L(1)20-(N)-(Fab')2
Br(1)40-(N)-(Fab')2
Br(2)40-(N)-(Fab')2

FIG. 63



(Fab')2s
(Fab')2
Br(2)- 40kD-(N)-Fab'2
Br(1)-40kD-(N)-Fab'2
L(4+5)-20kD-(N)-Fab'2
L(1)-20kD-(N)-Fab'2
MW markers
kDa
220_
130_
90_
70_
60_
40_
30_
kDa

FIG. 64

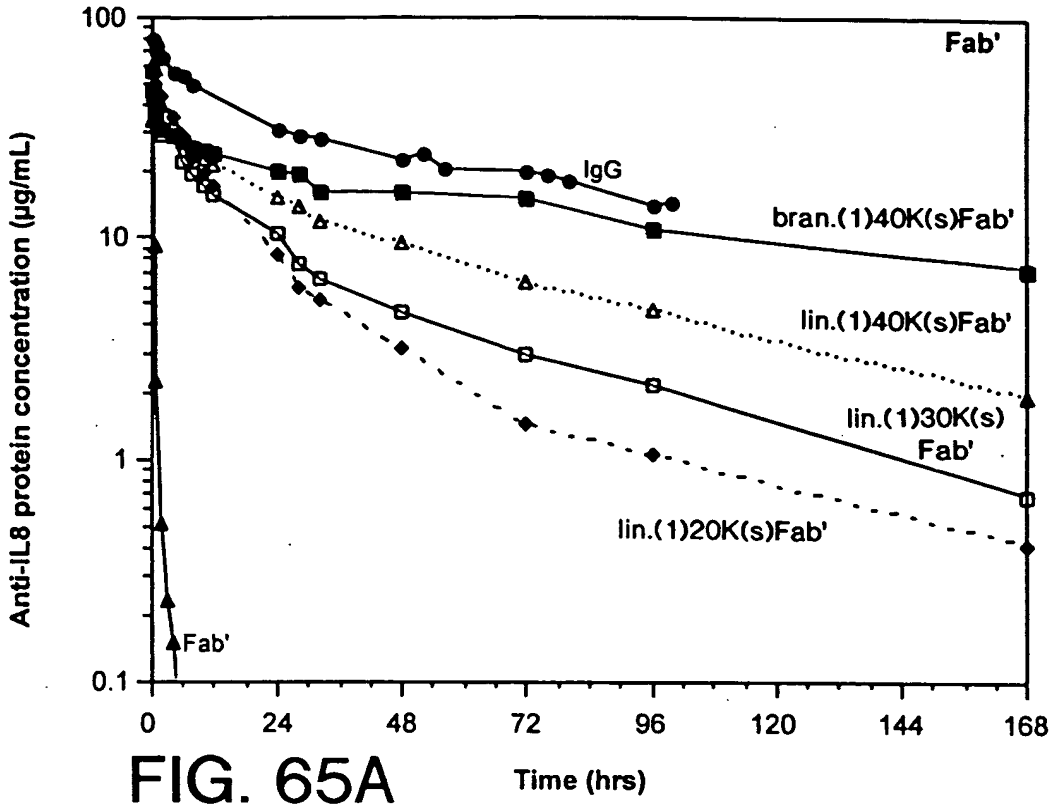


FIG. 65A

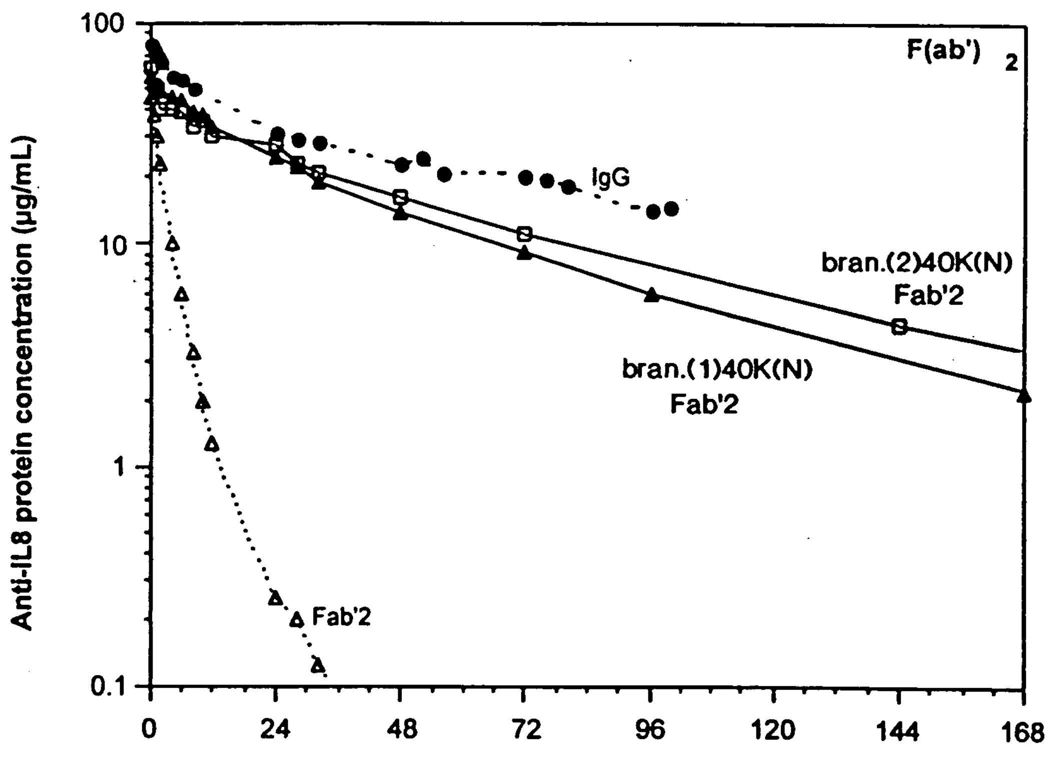
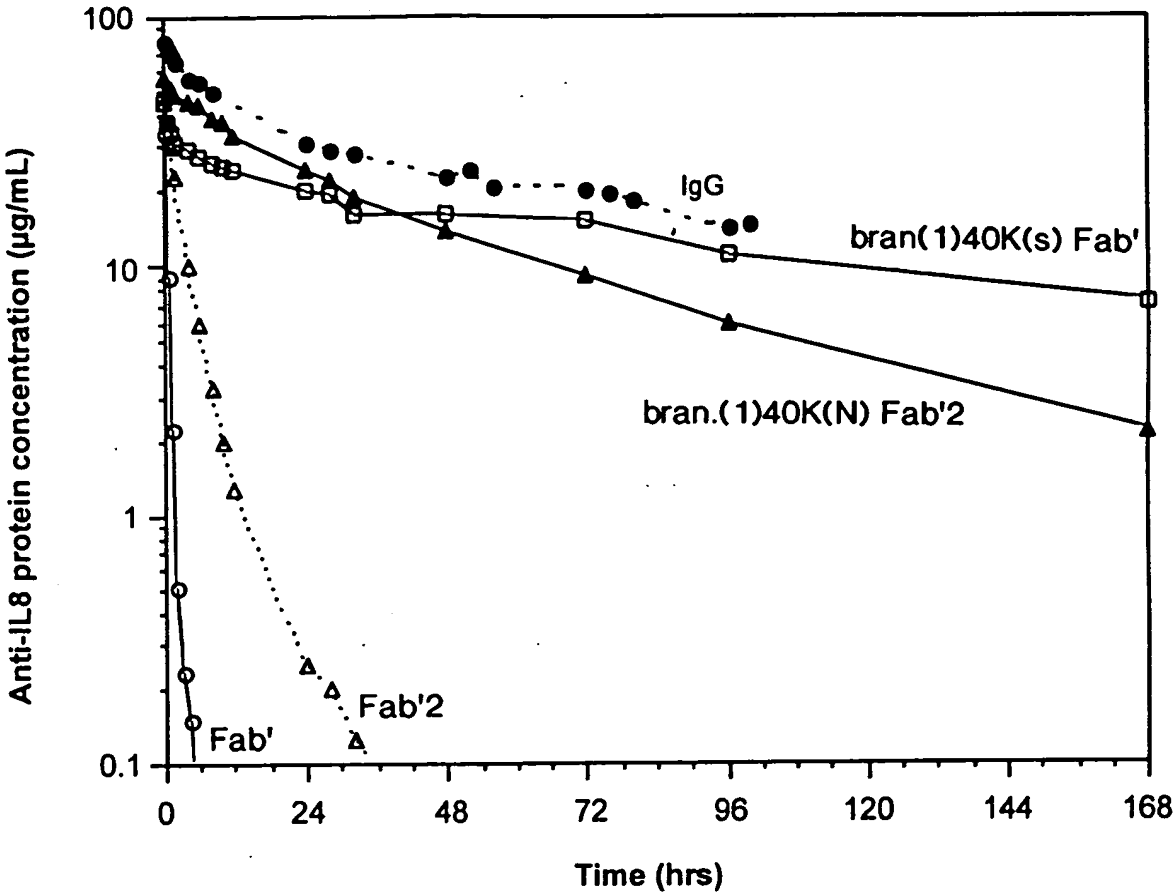


FIG. 65B



100
10
1
0.1
Anti-IL8 protein concentration (µg/mL)
0
24
48
72
96
120
144
168
Time (hrs)
IgG
bran(1)40K(s) Fab'
bran.(1)40K(N) Fab'2
Fab'2
Fab'

FIG. 66

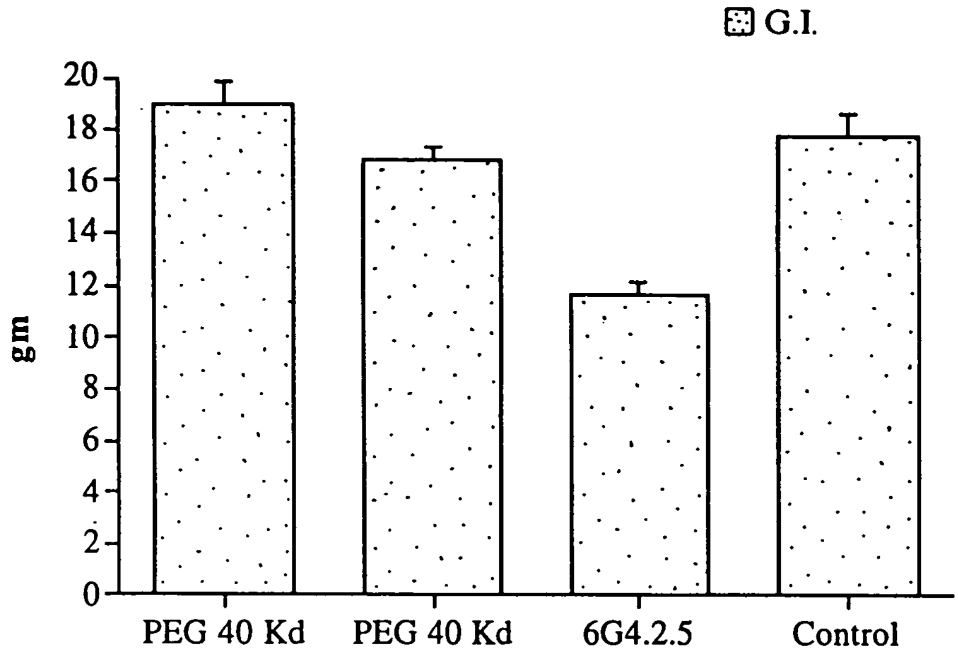

G.I.
gm
20
18
16
14
12
10
8
6
4
2
0
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 67

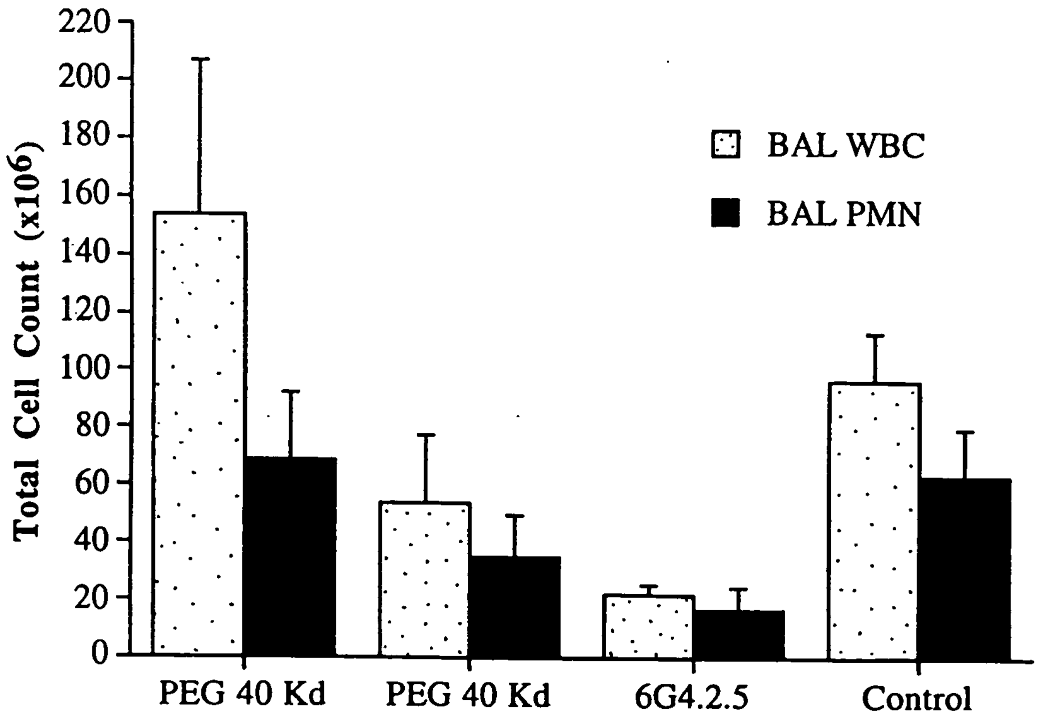

Total Cell Count (x106)
220
200
180
160
140
120
100
80
60
40
20
0
BAL WBC
BAL PMN
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 68

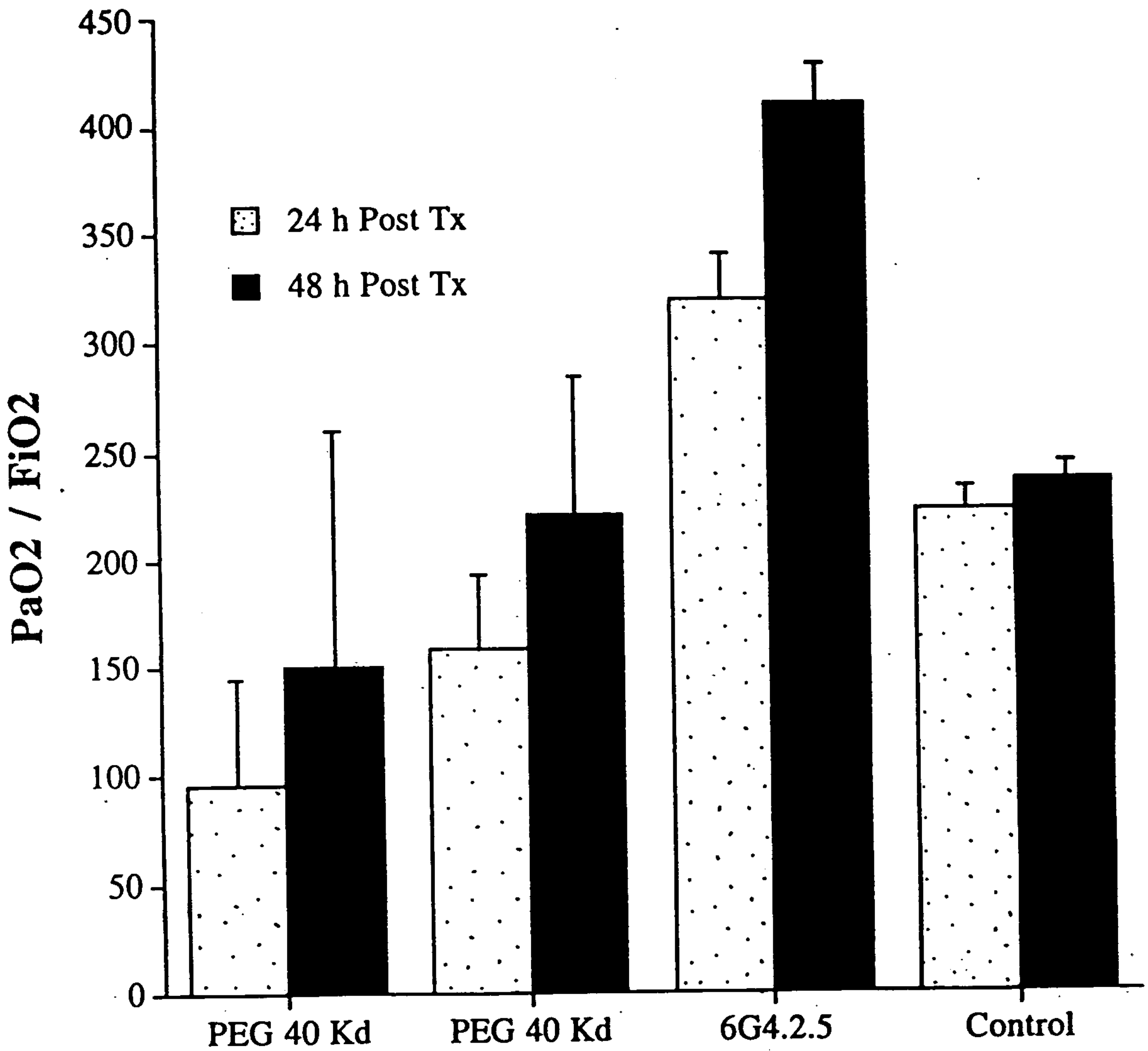
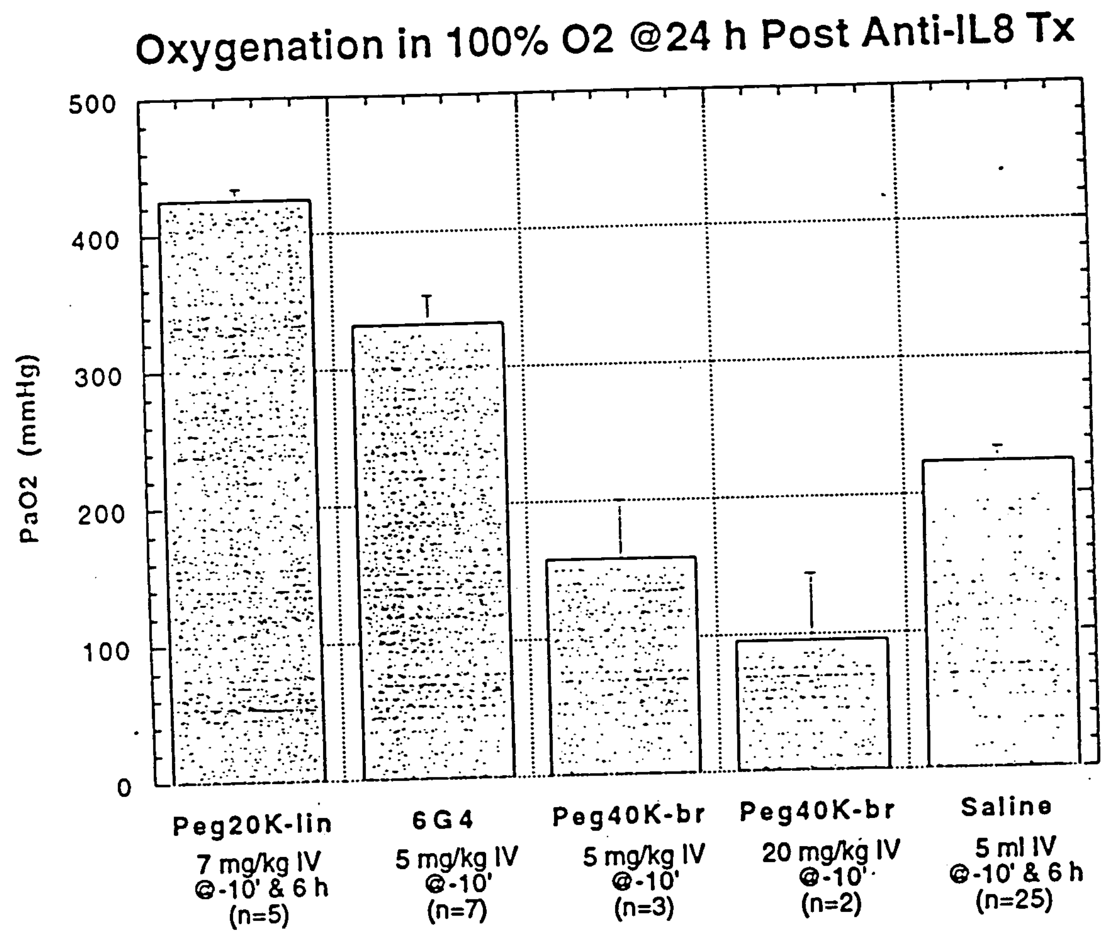

450
400
350
300
250
200
150
100
50
0
PaO2 / FiO2
24 h Post Tx
48 h Post Tx
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 69



Oxygenation in 100% O2 @24 h Post Anti-IL8 Tx
PaO2 (mmHg)
500
400
300
200
100
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=2)
Saline
5 ml IV
@-10' & 6 h
(n=25)

FIG. 70A

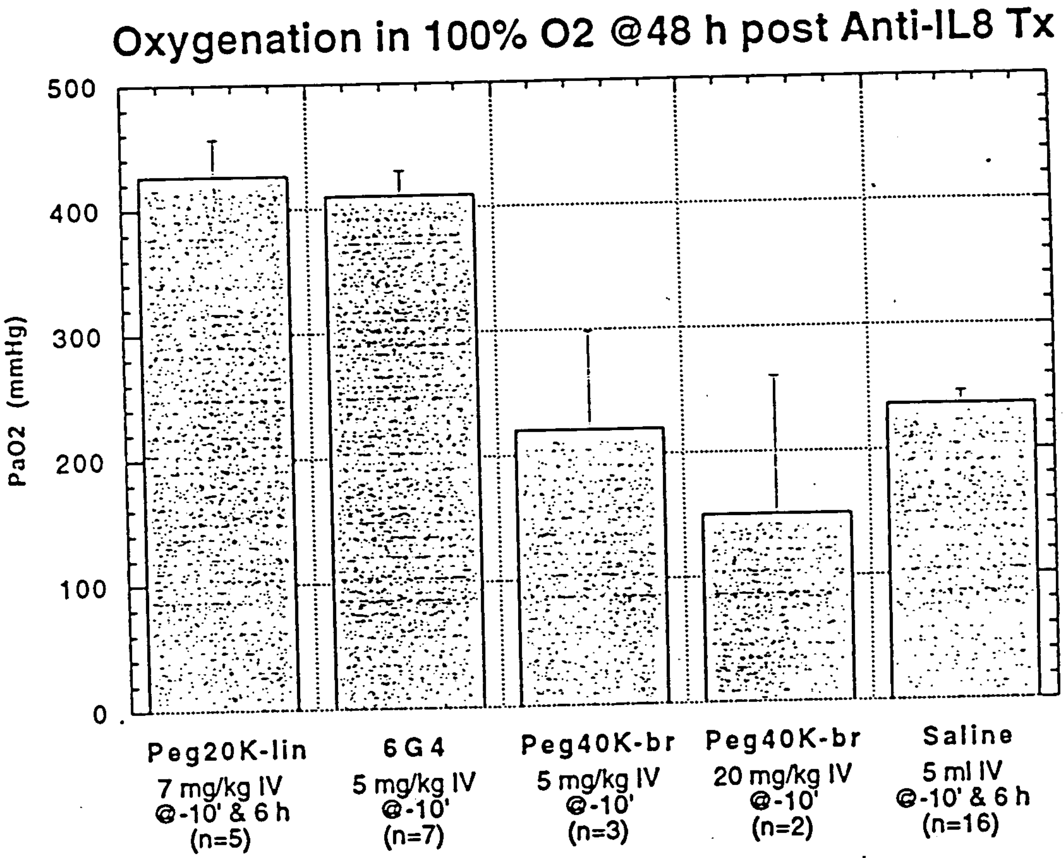

Oxygenation in 100% O2 @48 h post Anti-IL8 Tx
PaO2 (mmHg)
500
400
300
200
100
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=2)
Saline
5 ml IV
@-10' & 6 h
(n=16)

FIG. 70B

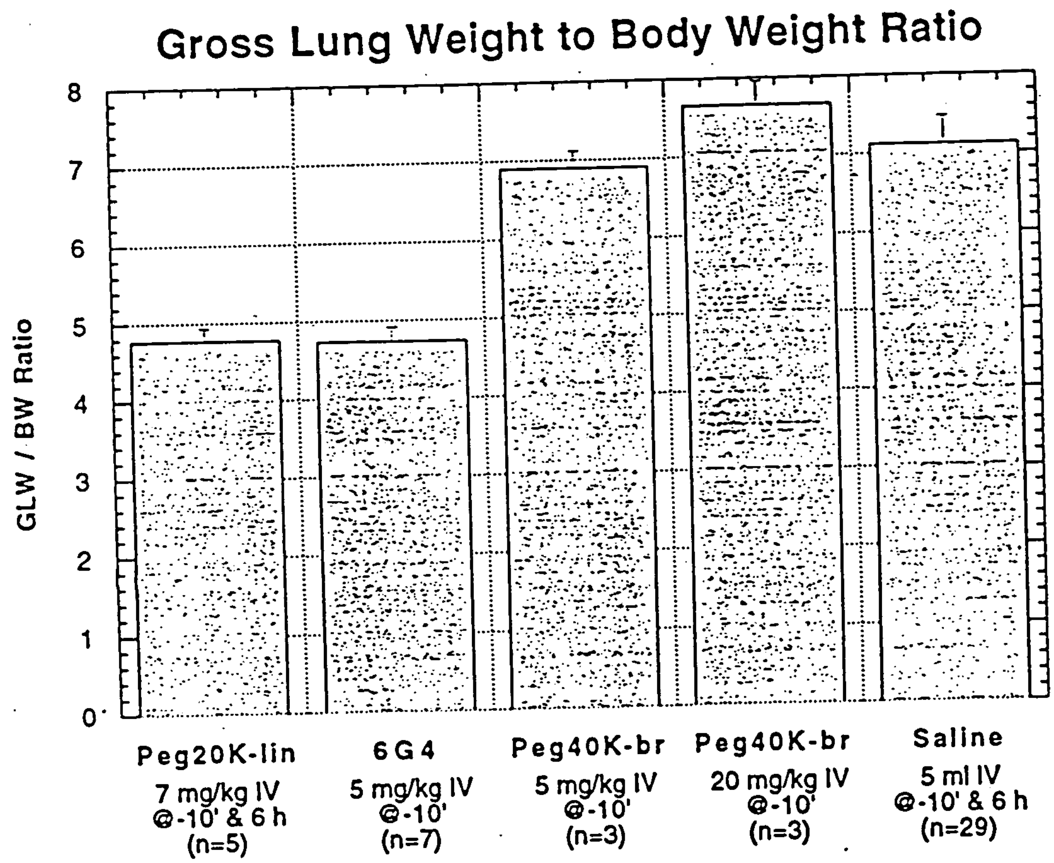
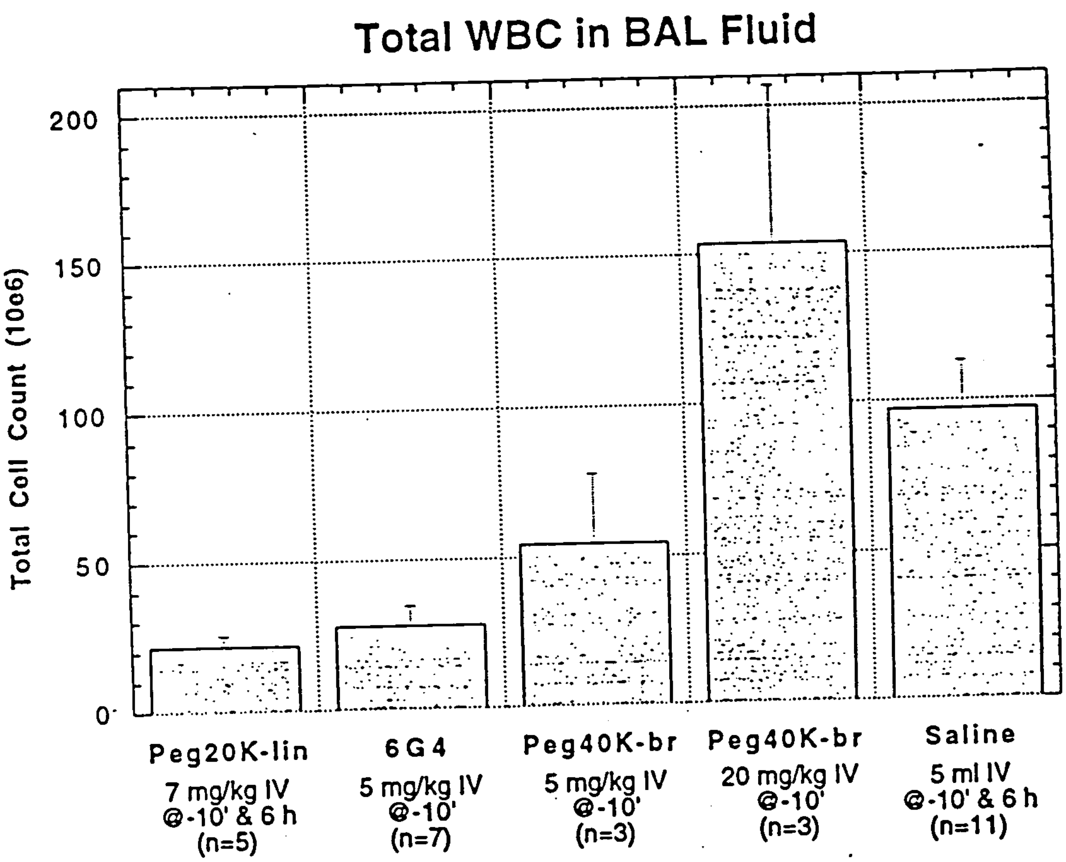

Gross Lung Weight to Body Weight Ratio
GLW / BW Ratio
8
7
6
5
4
3
2
1
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=3)
Saline
5 ml IV
@-10' & 6 h
(n=29)

FIG. 70C



Total WBC in BAL Fluid
Total Cell Count (10e6)
200
150
100
50
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=3)
Saline
5 ml IV
@-10' & 6 h
(n=11)

FIG. 70D

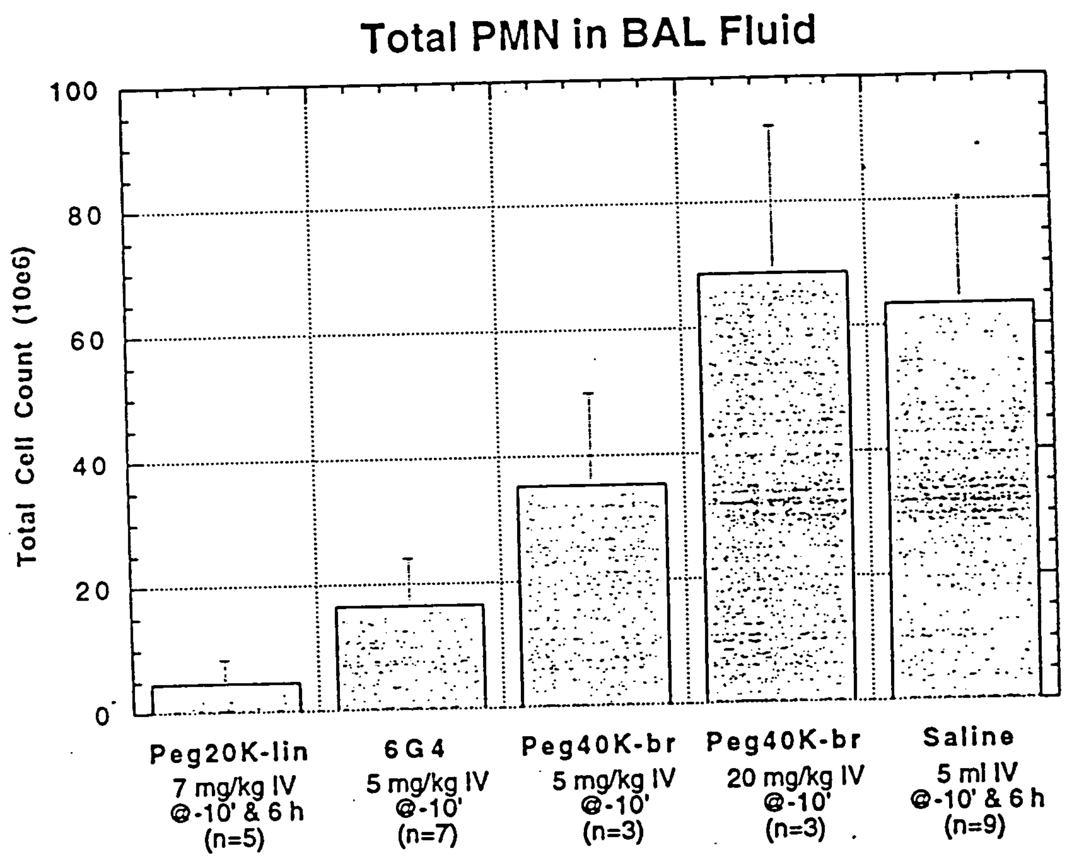

Total PMN in BAL Fluid
Total Cell Count (10e6)
100
80
60
40
20
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=3)
Saline
5 ml IV
@-10' & 6 h
(n=9)

FIG. 70E

The Effect of Pegylated Anti-IL-8 in the Rabbit Ear model of Ischemia-Reperfusion Injury

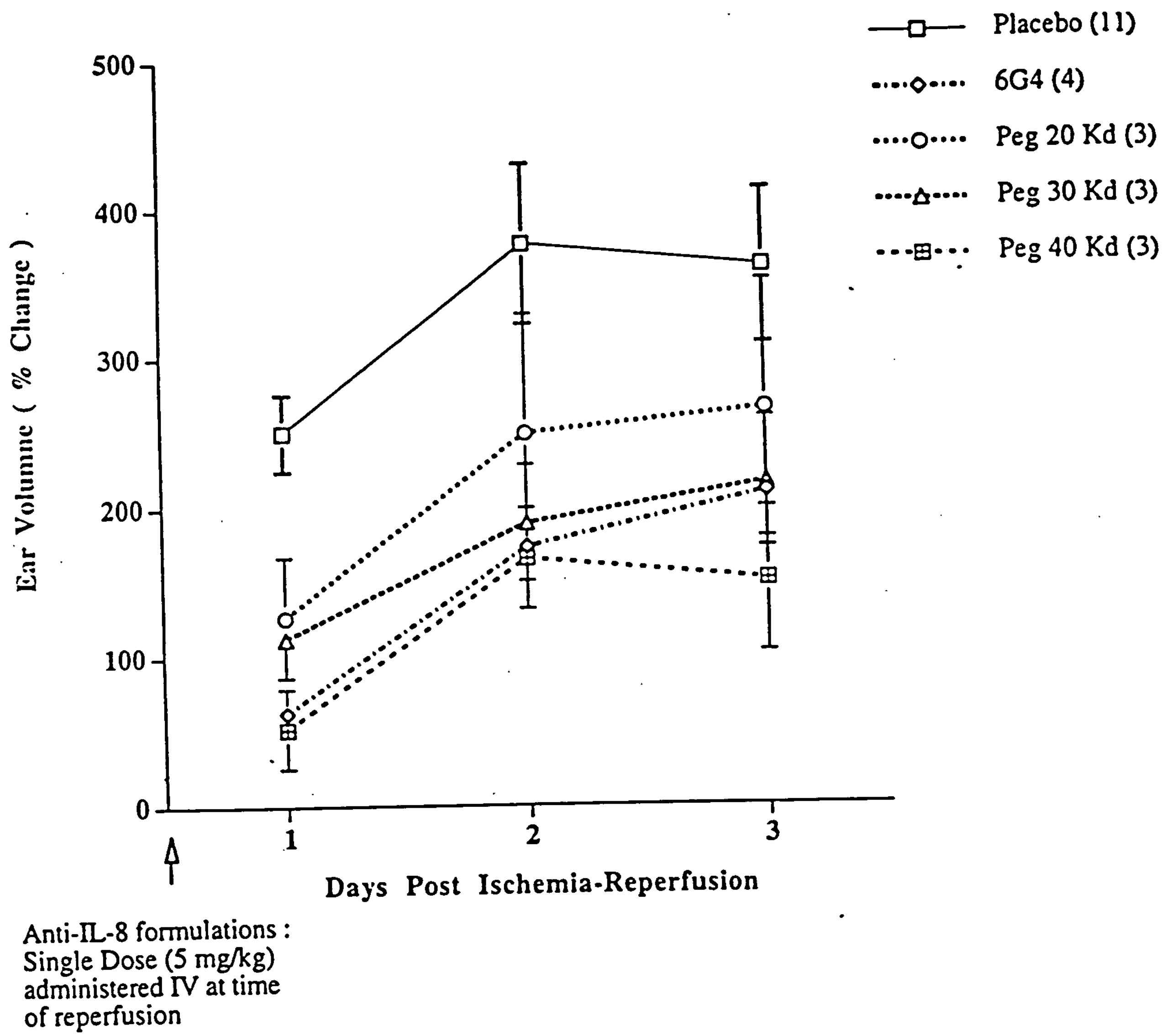


FIG. 71

Figure 72: 20K and 40K PEG rhuMAb VEGF Fab (approximately 3 mg/kg
Serum concentrations following IV administration

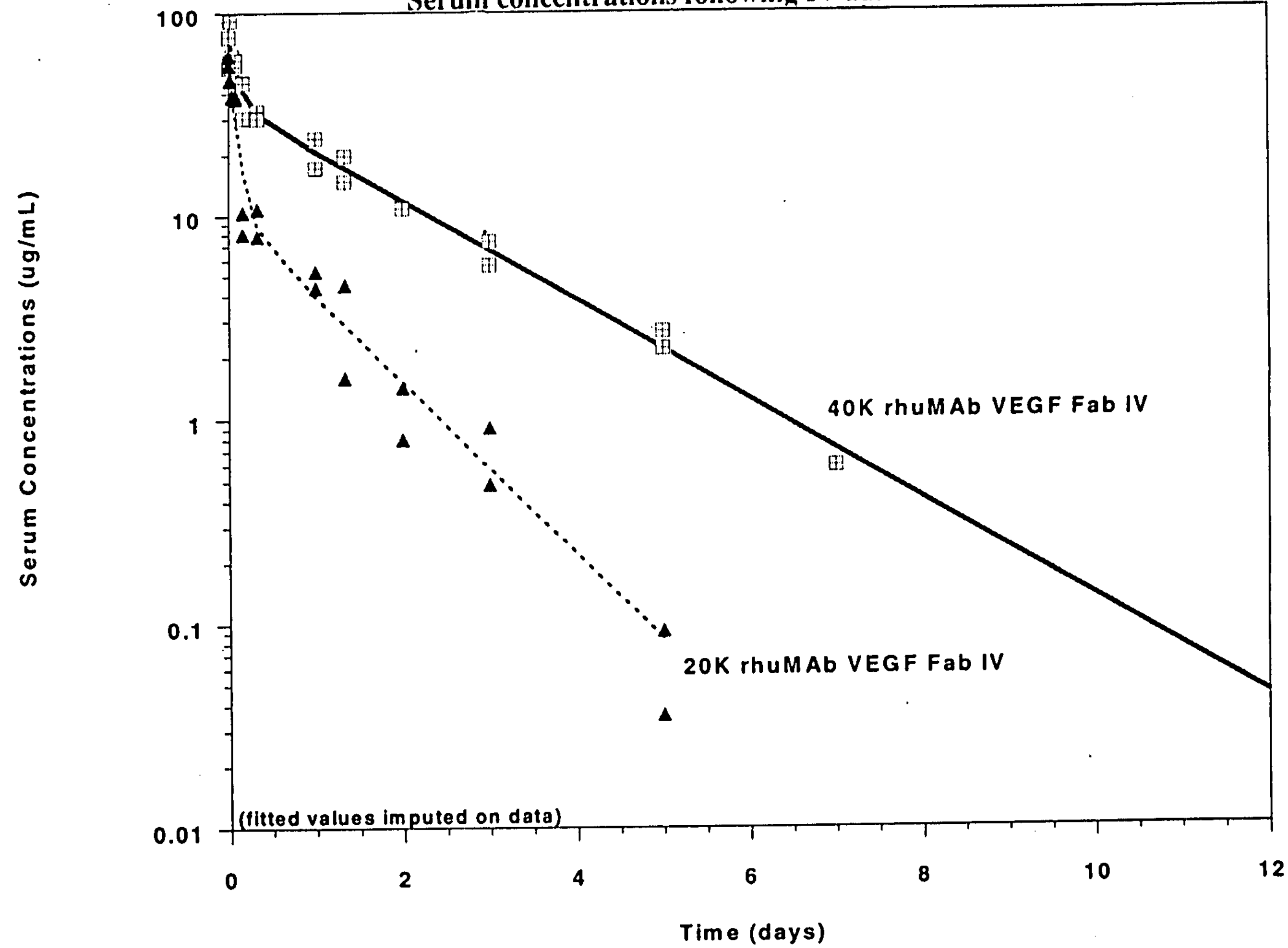
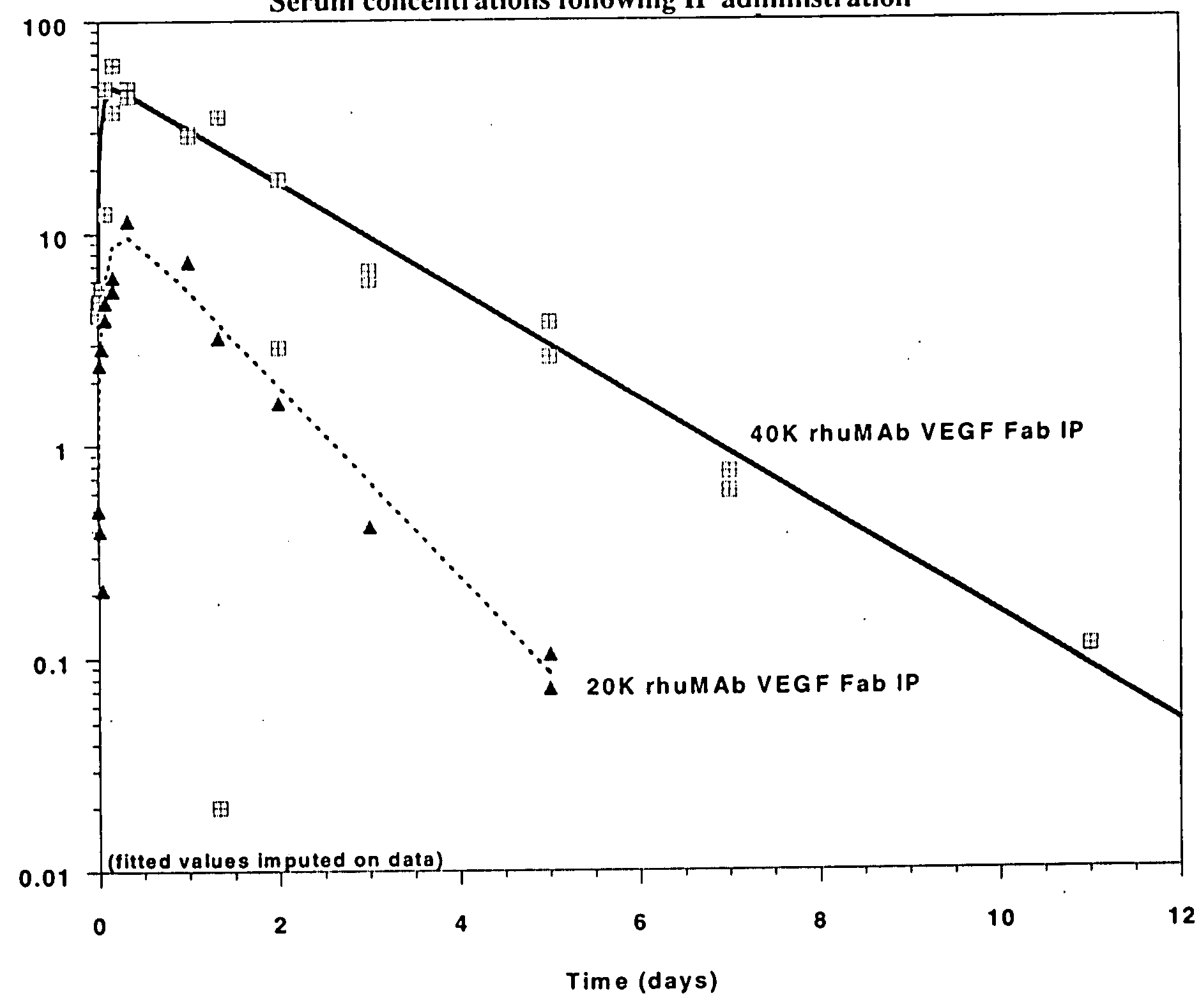
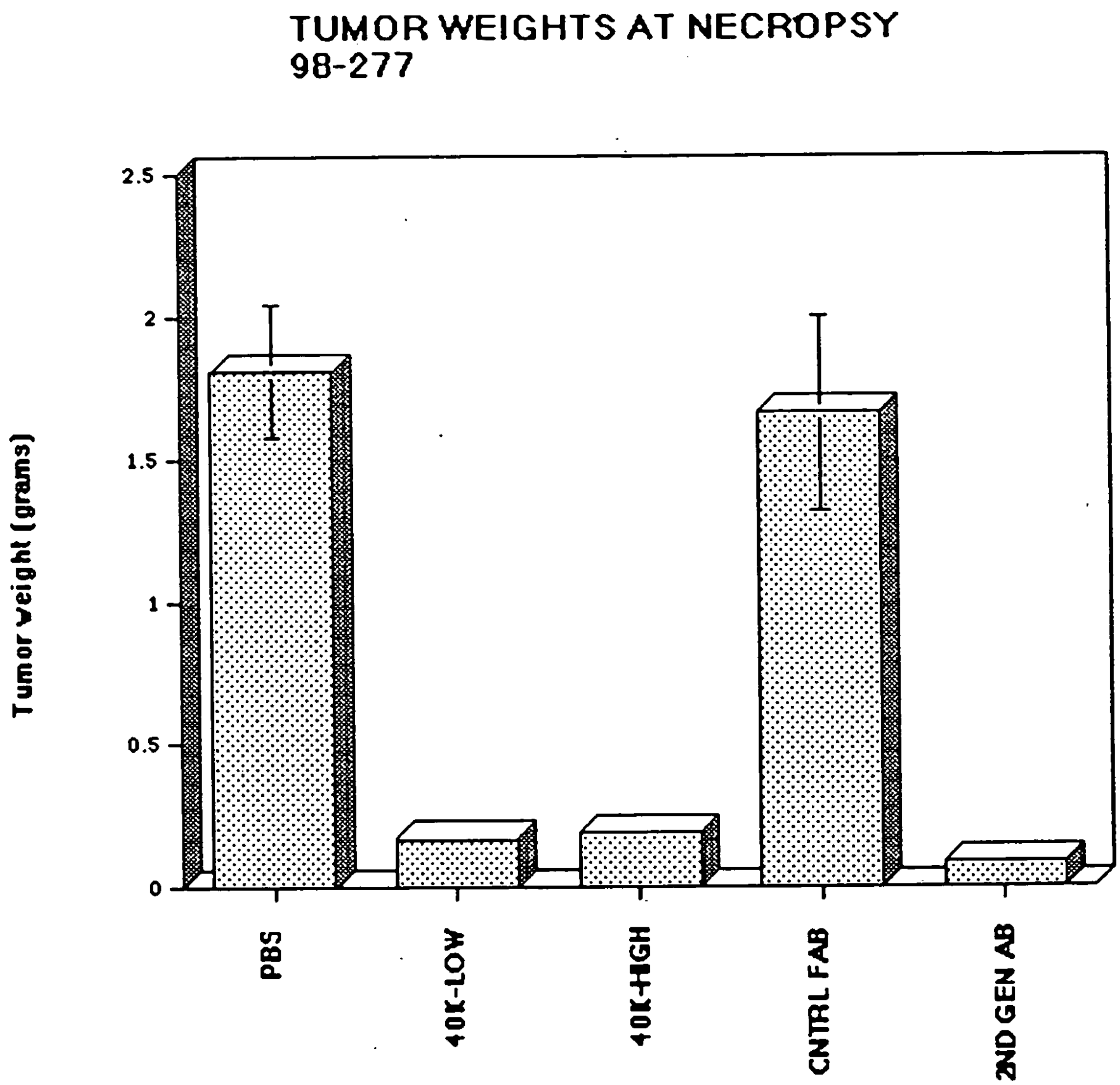


Figure 73: 20K and 40K PEG rhuMAb VEGF Fab (approximately 3 mg/kg
Serum concentrations following IP administration





TUMOR WEIGHTS AT NECROPSY
98-277
Tumor weight (grams)
2.5
2
1.5
1
0.5
0
PBS
40K-LOW
40K-HIGH
CNTRL FAB
2ND GEN AB

FIG. 74